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ERRATA

Page 41, paragraph 2, line 3, *read* Wakker *for* Walker.

Page 84, paragraph 1, line 1, *before* unshaded *insert* in.

Page 110, line 2, *change* rye, barley *to* those mentioned above and.

Page 219, table title and footnote 2, *read* Juglans *for* Jugulans.

Page 231, paragraph 3, line 3, and paragraph 4, line 7, *read* diethyldithiocarbamate *for* diethyldithiocarbonate.

Page 342, paragraph 2, line 7, *after* as *insert* by.

Page 472, series 37, and page 496, line 6 of text, *change* Kolotex *to* Kolodust.

Page 559, line 22, *change canavaline to canavaliae.*

Page 600, paragraph 3, line 2, *change* acropetal necrosis *to* stipplestreak.

Page 708, cuadro 2, *insert* heading, Trigos a 14 pares de cromosomas, for four last-named varieties, coordinate with heading, Trigos a 21 pares de cromosomas.

Page 714, line 7 from bottom, *read* "población" *for* "popblación."

Page 716, paragraph 5, line 1, *read* variabilidad *for* variabilidod.

Page 718, paragraph 2, line 2, *read* extremadamente *for* extremandamente.

Page 721, line 17 from bottom, *read* notar *for* notas; *read* trabajo *for* trabojo; line 3 from bottom, *substitute* Lineas susceptibles a la par que otras muy resistentes, se encontraron *for* en trigos "poblaciónes" tales como el Fulcaster, Gipsy, Harvest.

Page 722, paragraph 1, line 10, *read erythrospermum for esythrospermum.*

Page 964, paragraph 1, line 2, *read Champini for Chantini.*

Page 980, figure legend, line 5, *read* beat- *for* heat-.

Page 1133, table 1, column heading 4, *read* Number infected *for* Number inoculated.

Outside cover page, December, Contents, line 14; page 1181, title, paragraph 1, line 3, and paragraph 2, line 1; page 1183, paragraph 2, line 1, and paragraph 4, line 2; *change* malvaceara *to* malvacearum.

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WILLIAM ALLEN ORTON

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WILLIAM ALLEN ORTON
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LEWIS RALPH JONES

No loss from the ranks of American plant pathology has ever been personally mourned more widely and sincerely than that of Dr. W. A. Orton. Few had more varied friendships than his in Washington's scientific and administrative circles or more wide-reaching in those of the United States. Beyond our national confines his personal relations and professional correspondence encircled the globe. He was at once an internationalist of world-wide vision and interest and a friendly adviser to everyone who sought his aid. His breadth of interest and charm of personal qualities made him the helpful counselor for a host of foreign scientific visitors who made Washington the point of entry to American agricultural institutions. He was himself an experienced traveler and by skillful planning of itineraries and courteous letters of introduction he contributed much to the happiness and success of many such foreign guests.

Dr. Orton exemplified in a peculiar way the heroic in science. To those who knew his ancestry and early development this was not surprising. He came from the best Vermont stocks. As his name suggests, the blood of the Allens flowed in his veins. Since the days of "The Green Mountain Boys," this has stood for dauntless leadership, forgetful of personal dangers. The name of Orton also has long been known in New England science and administration as well as in pulpit and school. Born in a Vermont village, North Fairfax, he entered the Agricultural course in the University of Vermont when he was only sixteen and received his baccalaureate degree with honors (Phi Beta Kappa) at twenty. This was the better evidence of his native genius because from his childhood he was handicapped by partial deafness. As his botany teacher I well recall my impressions of this immature freshman always in the front seat as I lectured, eyes open, lips parted, eagerly "drinking in" every idea, although many of the words must have escaped him. I do not recall that he took a note but he always understood and retained. To the last weeks of life he showed remarkable powers of concentration upon any question under discussion, with unfailing ability to analyze and hold the essentials. In his student days, as in his later life

with its greater physical trials, instead of yielding to handicaps he forced them to contribute to his successes. If he recognized any limitations it was only that he might turn from lesser tasks to concentrate the more effectively upon the greater ones. Upon these he never admitted the possibility of failure.

For two years after graduation he remained at the University as an advanced student and research assistant. His chief interests concerned the parasitic fungi of Vermont, reviewing the literature, reworking all available material, collecting eagerly, and listing for publication. One of his University classmates of this period recalls a day when "Mr. Orton, an eager youth of somewhat indifferent college preparation, came into the laboratory saying that he had to use Latin books on mycology (Saccardo) although he had never studied Latin. To the admiration and chagrin of his fellow students who had spent several years upon Latin, young Orton acquired in a surprisingly short time a practical use of the language." He assisted also in our experimental work with potato diseases, inaugurating interests which guided him into important developments a decade later.

In 1899, when I was a guest in the laboratory of Dr. Erwin F. Smith in Washington, he told of his need of a promising research man for work on the control of cotton wilt. Immediately I urged consideration of young Orton, and he accepted my judgment. It certainly was something of a venture to accept for such a task a half-trained boy of twenty-two who had never even seen a cotton plant. With such brief suggestions as could be given in Washington, Orton went alone to spend the summer with the Carolina planters. He afterwards confessed his initial lonesome homesickness. But here, as always, his quick vision of the bigness of the job made him oblivious to all petty hindrances. His kindly personality, keen perception, sterling integrity, and tireless devotion to his tasks won the confidence and enduring friendship of the Carolina planters. From them he soon learned whatever their experience had taught them about cotton. Doctor Smith, following Atkinson's early work, had already diagnosed the cotton wilt as a soil-borne, vascular Fusarium disease. Seed disinfection and spraying were thus useless. Experiments with soil fungicides and fertilizers gave negative results. Hygienic measures, sanitation, and rotation, suggested by Smith, were only palliatives, pronounced impractical by the planters. What was to be done? Fortunately Orton had entered the old Division of Vegetable Physiology and Pathology, later merged into the Bureau of Plant Industry, at a time when the work was dominated by a small group of men with whom associations were most stimulating to thoughts along new lines. These included, in addition to Doctors Galloway, Woods, and Smith, such men as Carleton, Fairchild, Swingle, Waite, and Webber. Under such in-

fluences increasing attention was being given to the physiological aspects of phytopathological problems along with genetics and disease resistance in relation to control measures. Carleton had just brought from northern and eastern Europe his first large collection of foreign cereals as a basis for breeding for resistance to rust. Webber and Swingle were diffusing through the departmental ranks the glowing enthusiasm for plant improvements recently kindled by their epochal work in Florida on frost-resistant citrus. Orton was an eager young listener at such discussions. Almost immediately, as he surveyed the cotton fields of Carolina, he saw the possibilities of wilt control through disease resistance. Within two years he had the most remarkable evidence produced to that date as to the practical significance of disease resistance in plant-disease control. His first departmental publication, in 1900, "Wilt Disease of Cotton and Its Control," will remain a historic classic. This recorded the result of selections for disease resistance made the first summer and brought to completion after but two summers' work in the southern cotton fields by this inexperienced Vermont youth in his early twenties. Yet his conclusions are valid to-day. Along with these studies he had succeeded, where both Atkinson and Smith had earlier failed, in establishing, by pure-culture inoculation, the pathogenicity of this Fusarium on cotton. Nor was this all. His inspiration carried him on to the discovery of the wilt-resistant cowpea, which he, in association with Webber, showed to be the more remarkable in that it was also immune from the root-knot nematode. From this he turned to water-melon wilt, to overcome which he hybridized the resistant citron with the edible melon. These, with Bolley's work on flax, laid the secure foundation for all subsequent work with selection and breeding for resistance to the group of vascular Fusarium diseases. Thus in two years' time he mapped the way to the solution of problems which Smith had in 1898 defined as among the most seriously threatening in the field of phytopathology.[1]

The happy culmination of these earlier observations and researches upon disease resistance came with the formulation of his concepts as to the explanation and classification of disease-resistant types. These were outlined in the Yearbook of the Department of Agriculture for 1908 and in his address before the Conférence Internationale de Génétique, Paris, 1911.

His early work on these wilt diseases in the southern cotton and truck fields represents his outstanding personal researches. In these, except for brief association with Doctor Webber on cowpea breeding, he worked alone. Orton's genius was, however, always stimulated by contacts, and his natural instincts sought companionship.

[1] Smith, Erwin F. The fungus infestation of agricultural soils in the United States. Sci. Am. Supplement 48: 19981. 1899.

His unusual originality in ideas, coupled with his ability in organization for their advancement, was soon recognized in the Department of Agriculture. In 1907 he was made head of the newly created Office of Cotton, Truck, and Forage Crop Disease Investigations. Here his infectious enthusiasm found ampler field. He built up a scientific staff of some forty members, characterized at once by scientific ability, integrity in service, and devoted personal loyalty to their leader. He was a member of the committee of three which guided the organization of The American Phytopathological Society in 1908–09.[2] Of this Society he was a charter member and one of the drafters and signers of the articles of incorporation and held numerous offices, including the presidency and the editorship of PHYTOPATHOLOGY. At the first meeting of the Society it appointed a committee of five, of which he was a member, "to draft resolutions concerning wart disease of potato and white pine blister rust and to take steps to secure such action as would prevent their further introduction and spread." These two serious European diseases had recently appeared on the American Continent in quick succession in 1908 and 1909. From this, with correlated action by the American Association of Economic Entomologists, came the initiation of the Federal plant-quarantine service. The administration of this was placed in the hands of the Federal Horticultural Board, of which he was vice-chairman and pathologist for twelve years. These responsibilities stimulated his breadth of interest and information, especially concerning international problems. He was always a believer in the importance of international cooperation in quarantine matters. Whereas quarantines may naturally tend to create international frictions and material misunderstandings, he saw in them the opportunity for the very opposite. Might they not rather offer foci for international conferences and coordinated researches? To accomplish these he led in the movement, unfortunately interrupted by the World War, to encourage the visits to America of European pathologists and to favor like foreign journeyings of Americans for surveys and studies. The continuing benefits to international fellowship in scientific endeavor are to-day as clearly recognized in European countries as in America and Orton's early services in this field as fully appreciated.

[2] In addition to his official relations with The American Phytopathological Society he was also a charter member of the American Horticultural Society and held membership in other scientific organizations as follows: The American Association for the Advancement of Science, the Botanical Society of America, the Botanical Society of Washington, the Washington Academy of Sciences, the Society for Horticultural Science, the American Society of Agronomy, the American Forestry Association, the American Genetics Association, the American Society of Naturalists, the International Society of Soil Science, the Société de Pathologie Végétal, the Society of American Foresters, the Cuban Association of Sugar Cane Technologists, the International Society of Sugar Cane Technologists, the World Agricultural Society, the Cosmos Club, and Phi Beta Kappa.

His interest in potato wart and in the virus diseases of potato, together with the Conférence International de Génétique, took Orton to Europe in 1911. His earlier interest in potato pathology was thereby enhanced. His contacts with the work of Doctors Appel and Wollenweber in Germany were especially stimulating. Several important developments resulted. The system of potato-seed inspection and certification already in early operation in Germany under Appel's leadership was launched in America, where it continues as one of the great constructive preventive measures. Orton came back convinced of the importance of the virus problems in potato pathology. Through his publications and personal leadership he started the investigations concerning these problems which have ever since continued to increase in recognized scientific complexity and economic importance.

He brought back with him from Germany, as an addition to his staff, Dr. H. W. Wollenweber as Fusarium expert. This was done in order to put American studies of pathogenic Fusarium species into line with those of Europe, a type of contribution of continuing need with like complex problems. Later, he brought also Dr. Otto Appel from Germany that America might benefit from his experience with potato diseases in Germany. He contributed his influence in an important degree to the later visits of other leading European mycologists and pathologists, especially Butler, Cotton, Pethybridge, and Brierley, from Great Britain, Quanjer from Holland, Pole-Evans from South Africa, Foëx from France, and Vavilov and Jaczewski from Russia.

Dr. Orton organized and for several years carried on the plant-disease survey of the Bureau of Plant Industry, which has become of great importance in connection with work on the control of crop diseases. During the World War he was active in organizing research on diseases causing losses of vegetables in transit, market, and storage, which was developed in close cooperation with the food-products inspection service of the Bureau of Agricultural Economics and very materially increased knowledge of the causes of losses in shipment and marketing. As a result of this work methods for the reduction of disease losses in the process of marketing have been developed.

Following the war's interruptions of European international relations, Dr. Orton's vision sensed the increasing need for leadership in agricultural and especially phytopathological problems in the Latin Americas. The organization of our National Research Council gave him occasion for crystallization of these ideas. For nearly a decade, until his death, he was chairman of the Research Council's Committee on Phytopathology in the Tropics. Much thought was given to the correlation of tropical education

and research. Constructive reports were made on graduate education in tropical agriculture. The outstanding specific accomplishment was the organization under the National Research Council of the Tropical Plant Research Foundation. In 1924 he resigned from the Department of Agriculture to become the Scientific Director of this Foundation. This was designed as an agency to provide for tropical countries, particularly in the Western Hemisphere, a scientific service in support of crop production. As Director he gave concrete form to his ideals of such service through science in the interests of progress in two continents already closely interdependent. He always considered these undertakings a logical part of an inclusive national development. It is of interest to record that his most intimate early associates in inaugurating and advancing this project were Dr. George R. Lyman, then Secretary of The American Phytopathological Society, and Fred C. Meier, the present Secretary.

His first thoughts in specific researches dealt with the needs of large tropical-plant corporations such as the United Fruit Company. Such corporations engaged in any phase of plant culture in the Latin Americas are continually in need of reliable expert scientific advice upon pathological or other cultural problems. Their efforts at securing this by the temporary employment of individual expert advisers were, in general, expensive and relatively ineffective. He conceived the possibility of a non-profit-sharing organization under the patronage of the National Research Council, with expert scientific guidance, so functioning as to serve as an intermediary or activating agency in bringing the best scientific service to bear upon the peculiar problems involved.

His leadership of the Tropical Plant Research Foundation as Scientific Director continued until his death. This Foundation rendered expert services to such great corporate interests as the Cuba Sugar Club, representing the united sugar-cane growers of the Island, for which it organized and directed an experiment station. It served in an advisory capacity to various governments of Central and South America upon agricultural problems, including the organization and manning of both experiment stations and agricultural institutions. During the later years Doctor Orton was official agricultural adviser to the Pan-American Union.

In the field of tropical forestry Dr. Orton was a pioneer, giving it an impetus and a standing that it had never before enjoyed in the New World. His early grasp of the fact that in the American tropics the products of the forest are crops whose wise care and harvesting are as necessary as in agricultural crops led him to take a leading part in the little-known field of tropical forestry. An acceptance of silviculture and of conservative logging by the Latin-American Republics he conceived to be a part of his con-

tribution to their agricultural and economic stability. In this field he directed forest work in a number of Latin-American countries, and under his supervision the first survey of tropical American forest resources was made.

It must suffice to comment on only one other specific example, as outlined by Colonel George P. Ahern, formerly Director of the Philippine Forest Service, a Trustee of the Foundation representing forestry: "Dr. Orton at the invitation of the Brazilian Government in 1928 went to that country and discussed their forest problems with the officials. Before he left Brazil he submitted to them a draft of a forest policy so sound and comprehensive that it is marveled at by leading foresters, who are amazed that a man not a technically trained forester could prepare such a splendid document. This will, I believe, serve as a guide to the administration of the new forest service in Brazil for many years to come."

Much of this service was rendered gratuitously, and Doctor Orton in the best sense functioned as a missionary of Agricultural Science. Many expressions of appreciation of this followed Doctor Orton's death. One of these may be quoted from the *Revista de Agricultura de Puerto Rico* published in February, 1930, under the caption "*In Memoriam*:" "Porto Rico counted him among her practical benefactors. The cyclone of September, 1928, found him in our Island. After estimating the extent of the damage to our agriculture occasioned by this great disaster, Doctor Orton organized the work of rehabilitation, prepared reports for the Red Cross, and interested the Federal authorities in the defense of our agriculture, giving Porto Rico the benefit of the dynamic ability which always distinguished him."

All of this was done under increasing personal physical disabilities because of diabetes, which, added to his deafness, would have early inhibited a less courageous soul. Fortunately, these things never dimmed his intellectual vigor. It was chiefly a fight against malnutrition. Here again he forced his very disabilities to serve his scientific ends. Before the discovery of insulin he brought together for experimental culture in his own garden what was undoubtedly the most complete collection ever assembled of foliaceous food plants suited to a diabetic dietary. He mastered the physiological pathology of diabetes as well as the chemistry, culture, and cooking of these plants to a point where he was recognized as the national authority on the culture and use of diabetic foods of this class.[3] With the later use of insulin supplementing these dietary precautions and the wonderful co-

[3] Following Dr. Orton's death the eminent specialist on diabetes, Dr. Elliot P. Joslin, of Boston, wrote as follows: "When in my practice the outlook for diabetes was darkest, Doctor Orton brought the first rays of light. . . . From the very start his interest in diabetes was not confined to himself. He always tried to help some one else, and you know what a stimulus his garden, with its ninety-nine different diabetic vegetables, gave

operation of his wife and family, he fought his way back from the brink of bodily starvation to the amazing efficiency of the later years of his life.

For years his American friends, staff associates, and visiting agricultural scientists from all parts of the world found his home a welcome, grateful place of gathering. The memories will long remain with many of evening walks among his beloved dahlias and the inspiration that followed the free informal fellowship with men from many lands. Of these associations so pleasantly formed, not a few were later destined to continue as significant and productive friendships among students in kindred fields. One must number this great, unfailing gift of friendship in the long list of his contributions to mankind.

Any evaluation of his scientific accomplishments fails except as one recognizes the dominance of a great courageous spirit and a clear intellect over physical handicaps. It was this that made him so influential with and so beloved by all his immediate associates. As one of them writes: "He stood on an eminence apparently without realizing his own greatness. He was so friendly, so tolerant, so just, so modest with all, that it seems to me now we worked with a great man without then understanding his true greatness or the rare privilege of serving with him."

These words may well serve as a closing tribute to William Allen Orton, one of our heroes in science.

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PHYTOMONAS BETICOLA[1]

HARRY A. ELCOCK

INTRODUCTION

This paper presents the results of experiments carried on at the Michigan State College and at Rocky Ford, Colorado, from 1926 to 1928, inclusive, upon the root disease which has been called sugar-beet tuberculosis and which recently Brown (1) has called the bacterial-pocket disease. The investigations here presented deal with the nature and etiology of the disease and the effect of the infection upon the metabolism of the sugar beet. They include also studies on the reaction and affinites of the causal organism as well as observations and experiments to throw light upon the saprophytic existence of the organism in the soil.

The bacterial-pocket disease (*Phytomonas beticola* (Smith, Brown and Townsend) S. A. B.) is known only from the United States, and the reports of its occurrences are limited. The first material was received by the Pathological Laboratory, Bureau of Plant Industry, from Holly and Rocky Ford, Colorado, and Garden City, Kansas. Material used by Dr. Townsend came from Girard, Kansas. In 1923 it was found at Arlington Farm, Rosslyn, Virginia, by Brown (1). The disease was collected in 1926 by G. H. Coons and Dewey Stewart from a field at Lamar, Colorado, in which some hail injury had occurred. It was estimated that at least 10 per cent of the beets in certain portions of the field were affected. Since that time numerous collections have been made at Rocky Ford, Colorado, and other places in the Arkansas Valley, so that it can safely be said that the distribution of the disease is fairly general in this area. Mr. Asa C. Maxson, of the Great Western Sugar Company, has found the disease at Lowell, Wyoming. Extensive search has failed to disclose its occurrence in Michigan, although a number of cases of crown gall have been observed.

It seems then that the distribution of the bacterial-pocket disease is chiefly restricted to the western beet area, the single exception being at Rosslyn, Virginia. It also seems safe to predict that additional observations will show the disease more extensively distributed in the western territory than the few collections would indicate.

The occurrence of "galled" beets has been noted for many years, and these beets have always attracted attention at harvest time. Townsend (11)

[1] Acknowledgment is made to Michigan State College; Office of Sugar Plants, Bureau of Plant Industry; and to the South Dakota State College for facilities furnished for carrying on the work here reported. The writer wishes to acknowledge indebtedness to Dr. G. H. Coons for advice during the course of the work and for help in preparation of the manuscript.

states that when galls begin to appear on the beets in a given field they are at first few in number, increasing from year to year if beets are continued in that field. Speaking of overgrowths of the sugar beet in general, he further states that this type of disease of the sugar beet has increased rapidly in recent years and that it is still on the increase.

From the meager data at present available as to distribution of the bacterial-pocket disease of sugar beet as distinct from crown gall, it is obvious that no determination of actual loss caused by this disease can be made. The studies made in certain fields in Colorado indicate the potentialities of the bacterial-pocket disease. Surveys in various fields at Rocky Ford and Lamar, Colorado, have shown an average incidence of 3 to 4 per cent, while in one case in several large areas of the field, 10 per cent of the beets were affected. It is doubtful if the percentage of galled beets of the tubercle type in the general factory run approaches 1 per cent, but the high percentage found in certain fields indicates that under certain conditions the disease may become of increasing importance.

Losses from the bacterial-pocket disease of beets involve the following considerations: (a) Affected beets show marked tendency to rot (Fig. 1, C). The bacterial-pocket disease does not itself cause extensive necrosis of the affected sugar beet, but the rotting which occurs is mainly attributable to secondary invaders, chiefly saprophytic fungi. The ruptured epidermis and the more or less disintegrated nature of the gall tissue afford easy avenues of entrance for such organisms and, as a result, there is considerable decay of affected beets in storage piles. (b) The bacterial-pocket disease of the beet, as will be obvious from the photographs, causes loss because of the wasteful topping of diseased beets. Since the crowns contain such a high percentage of salts which interfere with the recovery of sucrose in the process of manufacture, the present topping practices necessitate removal of the crown just below the scars of the lowest whorl of leaves. The overgrowths of the bacterial-pocket disease are often on the crown and extend into the main part of the root (Fig. 1, A and B). This means a very large portion of the beet is cut off in the topping process, thus reducing the tonnage yield. (c) The bacterial-pocket disease of the beet, however, produces its losses very largely because of the profound upset of the sugar-beet metabolism which it causes, with the consequent reduction in sugar percentage and purity values. Townsend (11) called attention to the injurious effects that overgrowths produce upon the quality of the sugar-beet roots. He showed that the galls themselves are low in purity and are detrimental in the milling process. From his work it is not clear which type of gall was under observation.

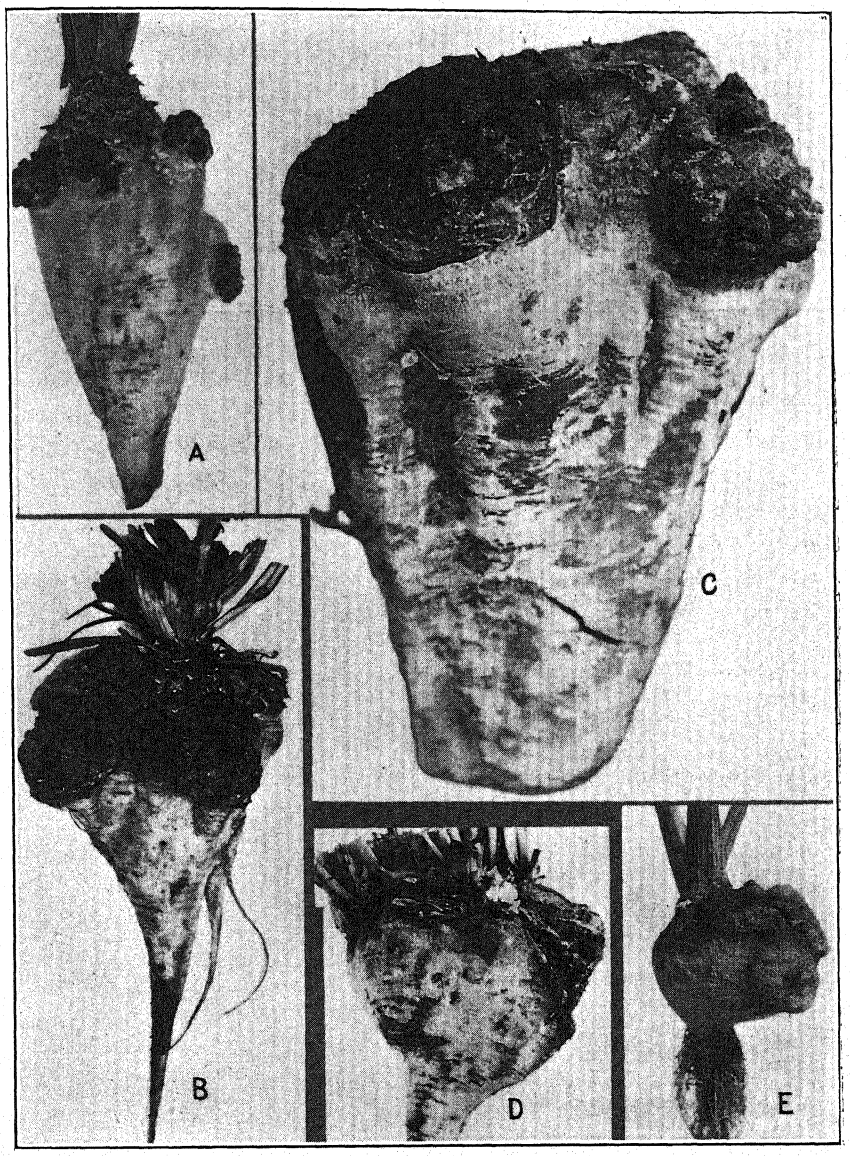


FIG. 1.—The bacterial-pocket canker of sugar beets caused by *Phytomonas beticola*. A and B. Typical overgrowths showing ridges which terminate in galls. C. Decay of older overgrowths. D. Bacterial-pocket cankers located at an injury made in cultivation. E. Gall on garden beet produced by inoculation with *Phytomonas beticola*.

EXPERIMENTAL WORK

In order to determine the extent of sugar percentage reduction and the effect upon the purity coefficient of sugar beets induced by the attack of *Phytomonas beticola,* diseased material collected in the field at Rocky Ford,

TABLE 1.—*Comparative analyses for sucrose and apparent purity of normal beets and of beets affected with the bacterial-pocket disease. Analyses made when beets were approximately two-thirds grown*

Condition of beets	Date 1927	Weight in pounds	Sugar in juice	Coefficient of apparent purity[a]	Size of over-growths[b]
1. Galled beet	Sept. 15	2¼	11.2	65.5	Small
Normal[c] "		2¼	12.8	77.7	"
2. Galled "	" 15	3¼	10.8		"
Normal "		3¾	12.0		"
3. Galled "	" 21	6	11.4		"
Normal "		4½	13.0		"
4. Galled "	" 25	2¼	10.2	68.0	"
Normal "		2½	13.6	78.4	"
5. Galled "	" 25	2¾	11.2		"
Normal "		3	11.1		"
6. Galled "	" 25	3	11.0		"
Normal "		4	12.6		"
7. Galled "	" 25	2½	11.0		"
Normal "		3¼	11.2		"
8. Galled "	" 15	2	10.8	63.0	Medium
Normal "		2¼	14.8	78.9	"
9. Galled "	" 15	3	10.4	64.5	"
Normal "		2½	13.0	78.3	"
10. Galled "	" 30	2½	10.4	66.2	"
Normal "		3	11.3	78.3	"
11. Galled "	" 25	3¼	11.2		"
Normal "		3¼	11.8		"
12. Galled "	" 15	4¼	9.4		Large
Normal "		3¾	13.0		"
13. Galled "	" 21	4	10.0	65.2	"
Normal "					
14. Galled "	" 21	2½	9.2		"
Normal "		2½	12.4		"
15. Galled "	" 30	2	11.6	72.0	"
Normal "		4¼	11.6	78.0	"
16. Galled "	" 25	4½	9.8	64.0	"
Normal "		3¾	12.3	79.3	"
17. Galled "	" 25	4	10.3		"
Normal "		4½	13.6		"
18. Galled "	" 25	6	9.2		"
Normal "		4½	10.8		"
19. Galled "	" 25	2½	10.6		"
Normal "		2½	13.2		"
20. Galled "	" 15	6	10.6	61.2	"
Normal "		4½	13.0	81.9	"

[a] Computed from solids in beet juice as determined by refractometer.

[b] Overgrowth in each case definitely determined to be due to *Phytomonas beticola.*

[c] The normal beet used as a check was adjacent to the galled one, except where a considerable difference in size made it desirable to choose some other beet within a radius of approximately 4 feet to serve as a check.

Colorado, was analyzed for sugar and purity. The results of these analyses were compared with parallel results obtained from normal beets which stood nearby in the row. The samples were taken from a field of commercial beets of a high sugar strain which were being sampled at weekly intervals for the purpose of determination of the course of sugar production during the season. Whenever a natural gall was found, a beet of approximately the same size was chosen from an area within a radius of 4 feet for a comparative test. The beets were analyzed by the usual individual-beet method (9) and before marked wilting had taken place. (Table 1.)

The sugar content and coefficient of apparent purity are the factors which determine the value of beets for sugar-making purposes. A considerable percentage of the total weight of the beets consists of soluble solids, of which sugar forms the largest portion. The galled beets show a very much lower coefficient of apparent purity due to the larger amounts of impurities in the form of soluble solids other than sugar. The greater the proportion of other soluble solids present the lower the purity, and from such beets it is more difficult to extract the sugar. These diseased beets with their low sugar and apparent purity percentage give syrup from which recovery of crystallizable sugar is poor, and the handling of such beets in the factory is unprofitable.

TABLE 2.—*Sugar percentages of diseased sugar beets contrasted with percentages found in normal beets. Tested at Rocky Ford, Colorado. Summer, 1927*

Variety	Sucrose in juice		Deviation from sucrose percentage of average check	Size of gall[a]
	Beets with overgrowth	Adjacent checks		
	12.5%	14.60%	−2.10	Small
50165–166	11.3	12.53	−1.23	"
	11.5	12.03	− .53	"
	11.0	12.69	−1.69	"
4088–23	10.0	10.84	− .84	Medium
	9.5	10.00	− .50	"
	10.0	10.53	− .53	"
	9.6	10.83	−1.23	"
	11.1	11.80	− .70	"
F. F.–24	9.8	12.82	−3.02	Large
	10.2	12.64	−2.44	"
	8.8	11.51	−2.71	"
	10.0	12.23	−2.23	"
	9.5	11.90	−2.40	"

[a] Gall in each case determined as due to *Phytomonas beticola.*

Another lot of beets was analyzed to furnish additional data on sugar-content relations. A number of artificially inoculated beets from plots of three different varieties were used. These beets were inoculated at intervals, so that on August 29, 1927, when the sugar determinations were made, there were three sizes of gall present, *i.e.*, small, medium, and large. The checks were, in the majority of cases, the two adjacent beets; however, if these did not furnish fair samples because of size, the checks were taken within a 4-foot radius. Attempt was made to choose the normal and diseased beets of as nearly the same size as possible.

In the data given in table 2, the figure for the diseased beet is the actual beet-sugar percentage found, and the check value is the average of the sucrose percentages of the two adjacent normal beets.

The analyses given in tables 1 and 2 are consistent in showing the marked reduction of quality of sugar beets brought about by the bacterial-pocket disease. Even in the cases where the overgrowths were small, there was a lower percentage of sugar.

SYMPTOMS

External characteristics: There are, as Smith, Brown, and Townsend (10) have pointed out, two distinct types of overgrowth occurring on the sugar-beet roots, and these have been designated as "tumors and tuberculosis" (11). The terms crown gall and tubercle or bacterial pocket seem preferable to designate the two types. In the field it is easy to confuse these types of overgrowth unless certain characteristics of the older galls are taken into consideration. In the bacterial-pocket disease of beets the young tubercle is a smooth, yellowish white proliferation that shows no sign of outer-cell disintegration. At this stage, the bacterial-pocket overgrowth might be confused with crown gall if internal structure were not taken into consideration (3). The older overgrowths in the bacterial-pocket disease are characterized by their roughness and strongly fissured surfaces. The diseased root generally shows a tendency to be conical or turnip-shape. That is, the beet does not develop with the normal, long, tapering root, but is short and very broad at the crown. Also, because of a marked excessive bud development, profusion of foliage may accompany this type of growth. The leaves at times come out in groups over the entire crown and often extend down to the ground line. Along with the flattening out of the crown, there generally arise swellings which extend as ridges from about the middle of the root up to the crown. These ridges generally terminate in a large rough overgrowth (Fig. 1, A, B, C.). The primary tubercular focus which is the cause of this ridge development is generally deep within the tissue of the root with secondary tubercles near the apex of the ridges. These aspects are in marked contrast to the condition in other beet-root galls, which gen-

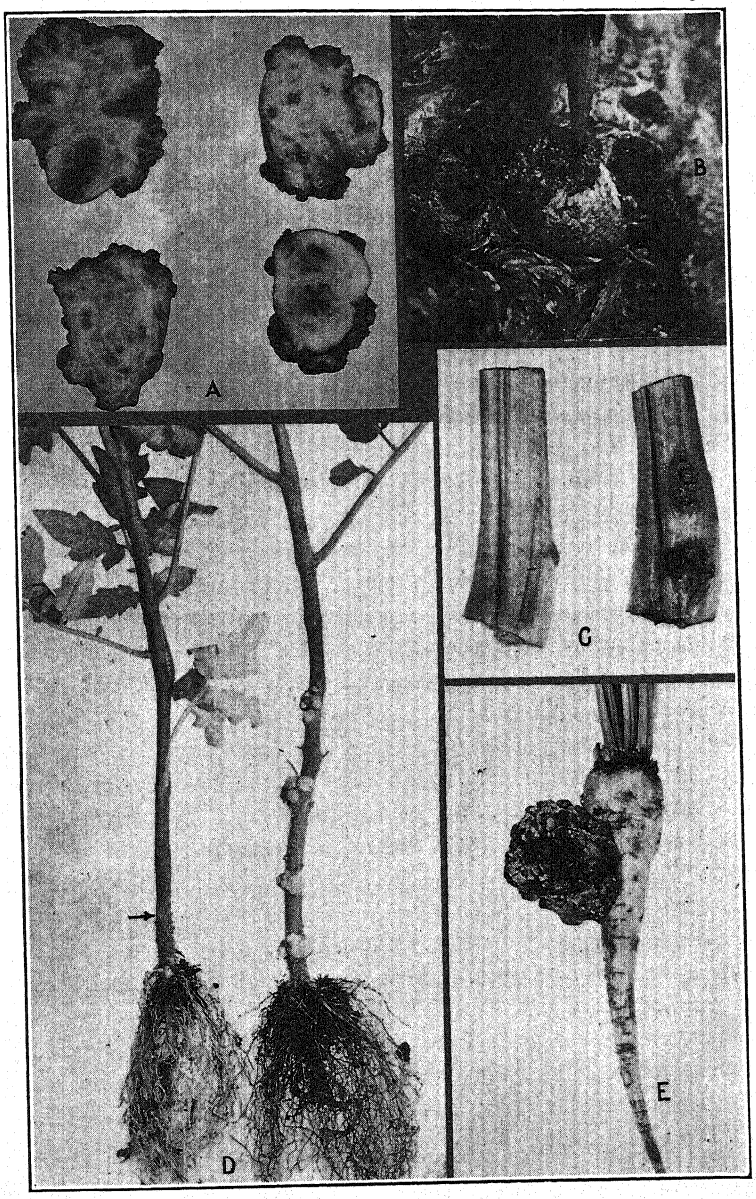


FIG. 2.—A. Bacterial foci characteristic of the pocket canker. B. Mass of bacteria exuding from a gall caused by *Phytomonas beticola*. (Photo by D. Stewart.) C. Galls produced by inoculation of sugar-beet petioles with *Phytomonas beticola*. (Wounded check on left.) D. Tomato plants inoculated with *Phytomonas beticola* (left) and *P. tumefaciens* (right). E. Crown gall of sugar beet caused by *Phytomonas tumefaciens*.

erally are lateral fleshy outgrowths upon the affected roots. In the case of the bacterial-pocket disease, secondary tubercles can be noticed on the surface of the primary tubercle, and these nodular growths, together with the deep fissuring, give the proliferation a very rough, warty appearance. Near the end of the growing season the tubercles show more or less decay of the outer layers of cells. The large fissures and cracks may penetrate the root tissue to a depth of an inch. These openings offer excellent avenues for entrance of secondary fungi, and these, under ordinary conditions, lead to partial decay of the crown (Fig. 1, C.).

Internal characteristics: The internal appearance of this disease is very distinct from that of crown gall. In the bacterial-pocket disease, localized, brownish, watery pockets occur (Fig. 2, A). These bacterial foci give point to the significance and applicability of the name tuberculosis, first applied to the disease. The gall tissues are a result of both hyperplastic and hypertrophic development. Microscopic examination of the brownish, watery spots or pockets, shows large masses of bacteria. These bacterial pockets vary from 2 mm. to 10 mm. in diameter and extend up and down the gall 1 mm. up to 3 to 4 cm. They are filled with material of a viscid consistency, which, under moist conditions, is sometimes so very mucilaginous that the bacterial slime when touched may be drawn out in sticky threads (Fig. 2, B). The diameter of the galls examined varied from about 1 cm. in young infections to as large as 1 dm. in old beets.

Petiole galls: Galls caused by *Phytomonas beticola* on leaf petioles were produced by artificial inoculations of the petioles but have not been seen under natural conditions. The proliferations on the petioles are at first small and appear to come from under the epidermis at the point of inoculation. As the gall becomes older, the epidermis begins to crack and small fissures are formed. The overgrowth then takes much the same rough appearance as the galls on the roots. The galls when young are green, but as the epidermis is ruptured the outer cells disintegrate, and become dry and brown (Fig. 2, C).

Since crown gall and the bacterial-pocket disease of the sugar beet are apt to be confused in reports on sugar-beet galls, the following table is given, which will enable fairly safe field diagnosis to be made. (Cf. Fig. 1, A, B, C, D, and Fig. 2, E.)

Relation of wounds to disease incidence: From field observations made in 1926 and 1927, in Colorado, it appears that the incidence of the bacterial-pocket disease in young, rapidly growing beets is strongly influenced by wounding, provided temperature and water relations are favorable for infection. The disease has been found associated with the wounding of beets

TABLE 3.—*Comparison of bacterial-pocket disease and crown gall of sugar beets*

Bacterial-pocket disease	Crown gall
A bacterial disease caused by *Phytomonas beticola.*	A bacterial disease caused by *Phytomonas tumefaciens.*
Comparison of Overgrowths	
1. Rough, warty, or tubercular overgrowth. Roughness due to rupturing of epidermis. Large fissures develop in the overgrowth.	1. Smooth or wrinkled overgrowth. Contour uneven in old galls, but epidermis usually intact without fissure formation.
2. Portion of root below the galls frequently ridged, ridges extending from middle of root to crown, terminating in the galled outgrowths.	2. Ridges absent; gall generally separated from root by stipe-like growth.
3. Misshapen, ridged, turnip-shape beet roots generally result from this disease, usually with a large amount of newly formed leaves coming out over entire crown.	3. Shape of root usually not changed except at place of tumor formation; no extra growth of leaves.
4. Secondary fungous invasion of the beet commonly prevalent.	4. No secondary fungous growth generally associated with this type of overgrowth.
5. In small, newly developed galls the contour may be uniform, the epidermis intact (later becoming cracked), flesh yellowish white, with numerous yellowish pockets.	5. In small newly formed galls, contour even, epidermis intact, ordinarily does not become fissured, flesh white.
Internal Symptoms of Roots	
1. Numerous yellowish, watery spots present within galls. (Bacterial pockets.) Surrounding tissue, white.	1. No bacterial pockets present; beet tissue, white.
2. Strands not very noticeable, outer cells of overgrowth decayed, fissures extending into tissue.	2. Strands of tissue extending in all directions within gall; outer cells, not decayed.

during cultural operations (Fig. 1, D). For example, mechanical injuries made by a cultivator, just before irrigation, in the case of a field where the disease had been prevalent the previous year led to infection of nearly 60 per cent of the wounded beets. Mr. A. W. Skuderna,[2] of the American Beet Sugar Company, has reported an instance in which an extremely high percentage of bacterial-pocket disease occurred following use of a heavy roller on a field when the beets were in the seedling stage. Crowns of beets often are injured by hail and such injuries have been observed to lead to abundant disease production in fields in southeastern Colorado. The hail storms generally are accompanied by heavy splashing rains which serve to carry the organism to the wounds as well as to provide favorable conditions for establishment of the pathogene. Mr. Asa C. Maxson, of the Great

[2] Verbal communication.

Western Sugar Company, Longmont, Colorado, has made similar observations: "Several times during the past 18 years I have found this type of growth very common in fields badly injured by hail.[3]

The following results of field inoculations made by the writer at Rocky Ford, Colorado, during the summer of 1927, are pertinent to the situations described. Broth cultures of three strains of *Phytomonas beticola* and one strain of *P. tumefaciens* were applied to exposed portions of the sugar and garden beets. In certain cases the inoculated areas were scarified by a few needle scratches, and in other cases the beets were left unwounded. Frequent examinations were made during the first 10 days, and 20 days after inoculation the beets were pulled and final readings taken (Table 4). In

TABLE 4.—*Inoculations with three strains of Phytomonas beticola (Nos. 1, 2, and 3) and P. tumefaciens (M. S. C. No. 146)*

Host	Organism and strains	Method of inoculation	No. of plants	No. of plants showing overgrowths			
				5 days	10 days	15 days	20 days
Sugar beet	Check	Wounded	12	0	0	0	0
" "	*P. beticola* 1	Wounded	25	0	10	12	24
" "	Check	Wounded	10	0	0	0	0
" "	*P. beticola* 3	Wounded	25	0	5	14	25
" "	*P. beticola* 3	Nonwounded	25	0	0	0	0
" "	Check	Nonwounded	25	0	0	0	0
" "	*P. beticola* 2	Wounded	25	0	0	0	0
Garden "	*P. beticola* 2	Wounded	25	0	3	4	9
Sugar "	*P. tumefaciens*	Wounded	25	0	0	0	15
" "	*P. tumefaciens*	Nonwounded	25	0	0	0	0

the case of two strains of *P. beticola* practically 100 per cent infection resulted in sugar beets. Inoculations without wounding failed in all cases; no galls appeared spontaneously on the check beets, wounded or nonwounded. Strain 2 of *P. beticola* was not pathogenic to sugar beets but did attack garden beets (Fig. 1, E). Crown gall was produced on 15 out of the 25 injured plants, and this figure doubtless would have been greater had a longer period been given for the galls to develop. From this test the significance of wounding to disease production is evident.

OVERWINTERING OF THE ORGANISM IN THE GALLS AND THE SOIL

In common agricultural practice, beets are topped in the field to the end that the débris containing *Phytomonas beticola* is scattered in the field.

[3] Letter to G. H. Coons.

Such galls on the surface of the soil would be subjected to drying and freezing, but the organisms within the galls would be more or less protected. To follow in a general way the effect of drying and low temperature upon masses of the organism in the galls, three tubercles from a beet that had been kept in the ice box (about 10° C.) from October 16, 1926, to January 15, 1927, were ground in a meat grinder. This material was mixed thoroughly with steam-sterilized soil from the greenhouse, having a pH reaction of 6.80. Ten pots were used and the following quantities of the ground material were added to the various pots of the series: 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 30.0, 50.0, 100.0, and 200.0 grams. Young sugar beets showing about 7 sets of leaves and whose root bundles had started to differentiate into the collateral type were transplanted, three plants to each pot. After the plants were firmly established, the dirt was removed from one side of the root. The crown and root were injured by scratching along the root with a sterile needle, so as to expose both the crown and lower portions of the beet to the material to be tested. The pots were watered uniformly with sterile water over a 27-day period. Thereafter, the plants received ordinary care. The results are given in table 5.

TABLE 5.—*Infections secured in a 40-day period with varying amounts of ground beet galls (Phytomonas beticola). Young sugar beets exposed to infection after being wounded at crown and along the roots*

Pot	Quantity	40 days	Galls produced
1	0.5 gm.	0	0
2	1.0 "	0	0
3	2.0 gms.	0	0
4	5.0 "	+	2
5	10.0 "	+	2
6	20.0 "	+	3
7	30.0 "	+	3 (4)[a]
8	50.0 "	+	3
9	100.0 "	+	3
10	200.0 "	+	3 (4)[a]

[a] Doubtful, *Phytomonas beticola* not recovered.

The persistence of the organism in galled beets which were stored at relatively low temperature and with considerable drying is shown by this test. The failure to secure infection with the smaller quantities of the ground sugar-beet gall indicates a reduction in the number of germs under the conditions, but the reduction probably is not significant in the field relations of the organism.

In many fields, in Colorado, fall plowing is practiced. Under such conditions the diseased material would be covered with soil. To determine whether the organism survives in the crowns when covered with soil, two beets showing galls were placed out of doors on November 3, 1926, and were buried in sandy loam soil at a depth of 6 inches. After being subjected to ordinary winter conditions in Michigan, with alternate freezing and thawing (lowest temperature −10° F.), one of these beets was examined March 3, 1927, and the other April 29, 1927, and both were found somewhat disintegrated. Isolations made from the interior of the decaying galls gave a great variety of organisms. From these a large number of yellow organisms were selected, some of which were shown by cultural tests and inoculations to be *Phytomonas beticola*. It is evident from this test that the bacteria in the galls buried in the soil can withstand severe winter conditions.

Aside from the persistence of *Phytomonas beticola* in the galls where more or less protection of the masses of bacteria is given by the beet tissue, it is evident that the organism may exist saprophytically in the soil as a free living form. In order to investigate this phase, the following preliminary test was carried on to test the technique of determination of suspected isolations by use of an agglutinating serum as proposed by Goldsworthy (4).

A composite broth mixture of *Phytomonas beticola* No. 1 and several other undetermined yellow organisms which had been isolated from soil at Rocky Ford, Colorado, was poured onto soil in flower pots in which young sugar-beet plants were growing. The beets were then punctured with a sterile needle and kept moist with sterile water; they later developed galls. Fourteen days after the suspension was poured on the soil, cultures were made from the soil in serial dilutions, as shown in table 6. The numerous yellow organisms which developed on the plates were isolated and grown on

TABLE 6.—*Test of a number of yellow organisms obtained from dilution plates of soil, against a serum agglutinating Phytomonas beticola at a titre of 1:1200*

Soil dilutions used	Yellow colonies selected	Number agglutinated by *P. beticola* serum
1/1,000	14	4
1/10,000	9	3
1/100,000	1	0
1/1,000,000	2	0
1/2,000,000	1	0
1/5,000,000	3	1

potato-dextrose agar and then tested with a *P. beticola* antiserum in the usual manner (Table 6).

It will be seen that a total of 8 organisms out of the 30 suspected isolations were agglutinated by the serum used.

These organisms were then used for inoculating rapidly growing sugar beets. One month after inoculation, seven of the eight organisms tested produced typical tubercles, thus confirming the preliminary diagnosis in seven cases out of eight. The organism failing to produce the disease in sugar beets may or may not have been *Phytomonas beticola.* The results of this test corroborate Goldsworthy's claim as to the value of agglutinating serum for quick diagnosis of suspects and indicate that suspected isolations from field soils could be readily diagnosed.

The method was then used in field tests. The first work was done with soil samples from a field at Lamar, Colorado, in which oats were growing and which, in the previous year, had shown considerable bacterial-pocket disease (10 per cent in certain portions). Twenty samples taken at random places in the field July 2, 1927, were used in the test. Serial dilutions of 1–10,000 to 1–2,000,000 were made in duplicate of each sample and these were employed in seeding potato-dextrose-agar (pH 7.56) plates. From these plates, 89 organisms showing the most typical pigment formation and growth characteristics were picked out and cultured. These 89 suspected organisms were then tested by use of the serum known to agglutinate *Phytomonas beticola* at a titre of 2200. Six showed a strong agglutination reaction with the test serum at a 1–1,000 dilution and were provisionally considered as being *P. beticola* (Table 7). The pathogenicity of each of the six organisms was then tested by wounding and inoculating rapidly growing sugar beets and garden beets in the experimental field. Many other beets were similarly wounded as a check on spontaneous occurrence of galls. No galls appeared on these latter beets. Five of the six organisms selected produced galls on sugar beets, and all of the organisms proved pathogenic to garden beets, thus confirming the preliminary diagnosis based upon the agglutination reaction. It is evident that *P. beticola* had persisted over winter in the soil under the dry conditions prevalent in the Colorado district, and the technique as outlined by Goldsworthy proved useful in field studies. Without using an excessive number of dilution plates, *P. beticola* was isolated from soil of a field where the bacterial-pocket disease had occurred on sugar beets the previous year.

Other soil samples taken from the various Rocky Ford, Colorado, fields where the bacterial-pocket disease was uncommon or where beets had not been grown at all recently yielded only one definitely identified culture of *Phytomonas beticola,* although many hundred platings were made. Irriga-

TABLE 7.—*Results of the agglutination test with Phytomonas beticola antiserum and organisms isolated from the twenty samples obtained at Lamar, Colorado. Record for colonies given as averages*

Soil dilutions used for plates	Plates		Serum dilutions				
	Number of colonies Average	Number of yellow colonies Average	Number of yellow colonies showing agglutination				
			1:5	1:50	1:100	1:500	1:1000
1/1,000	Too numerous	Too numerous					
1/1,000	" "	" "		None selected			
1/10,000	96	27	2	1	0	0	0
1/10,000	103	16	6	4	2	2	2[a]
1/50,000	47	22	2	1	1	1	1[b]
1/50,000	30	11	1	1	0	0	0
1/100,000	12	7	1	1	1	1	1[a]
1/100,000	17	3	1	?	?	?	1[a]
1/1,000,000	4	1	0	0	0	0	0
1/1,000,000	8	0	0	0	0	0	0
1/2,000,000	9	2	1	1	1	1	1[a]
1/2,000,000	5	0	0	0	0	0	0
Totals	331	89	14	9	5	5	6

[a] Produced galls on sugar beets and garden beets typical of *Phytomonas beticola.*

[b] Not pathogenic to suger beets, produced typical *Phytomonas beticola* galls in garden beet.

tion water from the Rocky Ford and Catlin systems[4] was tested by the above method, but in no cases were agglutinating organisms secured. Soil that as far as is known may be termed virgin soil gave no agglutinating organisms; however, it is probable that the amount of material tested was insufficient to prove that the organism is not present in that type of soil. No conclusion is warranted as to the natural occurrence of the organism in western soils, the one positive isolation being from a cultivated field on which beets may have been grown at some earlier time. It seems difficult to recover the organism except from fields with considerable infestation, and, if the organism is endemic in western soils, sugar-beet culture probably serves to intensify the infestation.

The above experiments, taken in conjunction with the overwintering results previously outlined, give evidence that *Phytomonas beticola* can overwinter in Colorado soils under ordinary climatic conditions. In the soil samples taken from the Colorado fields no recognizable fragments of

[4] Two irrigation systems supplying water to fields in the Arkansas Valley, Colorado. The water from the river is run onto fields at the upper end of the valley, and the waste water from these fields is used on the fields lower down the valley.

beets were included in the material plated, and the organisms are believed to have been free living forms. This evidence, coupled with the development of the disease here and there in the Arkansas Valley, leads the writer to the opinion that with this organism we have to do with a pathogene capable of living in the soil from season to season if not for longer periods.

EXPERIMENTAL WORK WITH STRAINS OF PHYTOMONAS BETICOLA

In the course of these studies a number of organisms have been isolated, tested for pathogenicity, and used in cultural and serological tests. These isolations have shown certain recognizable characteristics, and, for convenience, the organisms are discussed under the laboratory number. All conform closely to the cultural criteria for the species and have varied chiefly in pathogenicity, in rate of growth, and in intensity of pigment.

No. 1 was isolated from a sugar beet received from Rocky Ford, Colorado, October 16, 1926. It produced abundant growth in media and was strongly virulent in a series of inoculation tests.

No. 1-a was reisolated from a sugar beet inoculated with strain No. 1. The organism made good growth on laboratory media and showed strong virulence for sugar and garden beets as well as Swiss chard. "R" types were profuse.

No. 1-aa was reisolated from a sugar beet artificially inoculated with No. 1-a. Reactions were the same as for No. 1-a, but R and "S" type colonies were present in about equal numbers.

No. 2 was isolated by Mrs. M. C. Strong, of Michigan State College, from a beet sent from Lamar, Colorado, October 13, 1926. This organism produced good growth on laboratory media. It was distinct from many other strains in showing strong virulence on garden beets but was not virulent to sugar beets in repeated inoculation tests.

No. 2-a was reisolated during the summer of 1928 from a garden beet at Rocky Ford, Colorado, which had been inoculated with strain No. 2. This organism, like strain 2, was virulent to garden beets, but nonvirulent to sugar beets in repeated tests.

No. 3 was isolated from soil obtained at Lamar, Colorado, as described in a previous section. The organism produced good growth on laboratory media and exhibited strong virulence to both sugar and garden beets.

Phytomonas tumefaciens, M. S. C. No. 146. A culture obtained from Dr. E. F. Smith, of the Laboratory of Plant Pathology, U. S. Department of Agriculture, and labelled "Hop Strain," has been maintained in stock culture for some years at Michigan State College, and was used in comparison with *Phytomonas beticola.*

The recent work published by Brown (1) as to cultural work, pathogenicity, and host considerations make unnecessary extended record of

findings in cultural studies with this organism. The standard cultural tests were used and, in every important particular, similar and confirmatory results were obtained. The characterization of *Phytomonas beticola* as given by Miss Brown seems adequate to permit sure diagnosis and differentiation.

An abridged statement as to pathogenicity, which also confirms Miss Brown's findings, may be given: The cultivated sugar beet, *Beta vulgaris* L., as represented by two varieties grown commercially at Rocky Ford, Colorado, namely, Pioneer and Flat Foliage, were found very susceptible to the bacterial-pocket disease discussed in this paper. No varietal differences as to resistance or susceptibility were found in sixteen varieties of commercial beets which were tested by artificial inoculation. Typical overgrowths also have been produced on garden beets, *B. vulgaris,* and Swiss chard, *B. vulgaris cicla* L. When inoculations were made into tomatoes, *Lycopersicon esculentum* Mill., no typical gall formation resulted. However, a small, smooth, yellowish spot on the stem at the point of injection (Fig. 2, D, left) was noticed 28 days after inoculation. Pure cultures of the organism were obtained from this spot at that time. It is probable that the tomato merely harbored the organism at the place of inoculation without definite invasion occurring. Tobacco, raspberry, apple, and geranium plants were inoculated but showed no symptoms of gall formation. In contrast to these results, all the above-mentioned plants with the exception of tobacco developed overgrowths when inoculated with the crown-gall organism in a series of tests paralleling the above-mentioned inoculations (Fig. 2, D, right). No wild host for the organism has been found, although various weeds belonging to the family Chenopodiaceae or closely related families were examined in fields where the disease was developing in considerable amount on the sugar beet.

SEROLOGICAL TESTS WITH THE VARIOUS STRAINS OF PHYTOMONAS BETICOLA AND OTHER BACTERIAL PATHOGENES

Agglutination Tests. Several writers have reported use of specific agglutinating sera as a method of identification of phytopathogenic bacteria (6, 7, 8). The writer's studies have been concerned with the production of specific agglutinating sera for *Phytomonas beticola,* for use in diagnosis of suspected organisms and in comparative tests in which other plant pathogenes were included. The methods followed were, in general, similar to those given by Zinsser *et al.* (12) and Sharp (8), but it was found, however, that if dead or heat-inactivated *P. beticola* was used as antigenes the sera obtained were not of so high a titre as was later obtained by using the living organisms. The organisms used as antigenes consisted of various

strains of *P. beticola,* a culture of *P. tumefaciens* (Hop Strain from E. F. Smith), and a culture of *Erwinia carotovora* (heated). Forty-eight-hour-old cultures on potato-dextrose agar (pH 7.2) were washed from the slant with normal saline, and, after filtering through sterile cotton and shaken with beads, were adjusted to a standard density (No. 2) by use of a McFarland nephelometer.

In the first attempts the standardized suspensions of *Phytomonas beticola* were heated to 56° C. for one hour before the intravenous injections. The titres obtained with this material were not very high, namely, 320 and 640. In another series of rabbits the antigene was injected without the preliminary heating. After 5 intravenous injections of 1 cc. of the antigene at 2- to 4-day intervals, sera of much higher titre (2200 to 3600) were obtained, as shown in the table.

Having obtained the high-titre sera, various plant and soil bacteria were subjected to cross-agglutination tests in which the macroscopic method of determination was used. Difficulty was experienced with *Phytomonas tumefaciens* because of its tendency to form small clumps spontaneously in the 0.85 per cent salt solution (*cf.* Link and Link, 6). With a suspension in 0.5 per cent salt solution centrifugalized to remove the heavy clumps, a fairly stable suspension was obtained.

TABLE 8.—*Antisera from rabbits used in agglutination tests: 5 injections, 1 cc. each, of nonheated bacterial suspension, at 2- to 4-day intervals were given*

Serum No.	Antigene	Method	Titre obtained
407[a]	*Phytomonas beticola* No. 1	Intravenous	1200
239	" " " 1-a		2200
101	" *tumefaciens* " 146	"	2750
397	" " " 146	"	3050
294	" *beticola* " 1-a		3250
295	" " " 2	"	3600
395	*Erwinia carotovora*	Intraperitoneal	640

[a] Sera were given a number corresponding to the animal number at Michigan State College.

In table 8 the results of the agglutination tests are given. In each case, the antiserum was used with its homologous antigene, and in all cases except with the *Erwinia carotovora* serum, which was known to be of low titre, strong flocculation in dilutions up to 1 to 640 was obtained. When other strains or species were used, the agglutination was either much weaker or

TABLE 9.—*Results of agglutination tests in which the various antisera and a number of bacterial antigenes were employed*

Antigene	Antiserum 239, homologous with *Phytomonas beticola* No. 1-a							Antiserum 397, homologous with *Phytomonas tumefaciens* No. 146							Antiserum 395, homologous with *Erwinia carotovora*						
	Dilutions							Dilutions							Dilutions						
	1/10	1/20	1/40	1/160	1/320	1/640	Check	1/10	1/20	1/40	1/160	1/320	1/640	Check	1/10	1/20	1/40	1/160	1/320	1/640	Che
P. beticola No. 1-a	++++	++++	++++	++++	++++	++++	−	+	+	−	−	−	−	−	±	−	−	−	−	−	−
P. beticola No. 2-a	+	+	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
P. beticola No. 2	+	+	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−
B. noctuarum[a]	−	−	−	−	−	−	−	−	−	−	−	−	−	−							
E. atroseptica (1)	−	−	−	−	−	−	−	−	−	−	−	−	−	−							
E. atroseptica (2)	−	−	−	−	−	−	−	−	−	−	−	−	−	−							
P. vignae	+	±	−	−	−	−	−	+++	+	++	±	−	−	−	−	−	−	−	−	−	−
B. sphingidis[a]	−	−	−	−	−	−	−	−	−	−	−	−	−	−							
E. carotovora	±	−	−	−	−	−	−	−	−	−	−	−	−	−	++++	++++	++++	++++	++++	++	−
P. tumefaciens	±	±	−	−	−	−	−	++++	++++	++++	++++	++++	++++	−	+++	++	++	±	−	−	−

++++ Strong flocculations.
+++ Medium strong flocculations.
++ Medium flocculation.
+ Slight flocculation.
± Doubtful flocculation.
− No flocculation.
[a] Pathogenic to insects, obtained from G. F. White.

absent, the differences being so pronounced as to indicate clearly specificity of reaction. The following reactions deserve comment.

The antisera, 239 and 294, obtained by using *Phytomonas beticola* No. 1-a as an antigene, showed no strong reaction with any antigene except the homologous one (Tables 9 and 10). However, strains of *P. beticola* No. 2 and 2-a, isolated from typical bacterial-pocket galls on beets grown at Lamar and Rocky Ford, Colorado, respectively, showed slight flocculation in a dilution of 1–20. *Erwinia carotovora* showed a very slight tendency toward a reaction, but agglutination could be detected only with micro-agglutination methods. *Phytomonas vignae* showed a slight reaction with serum from rabbit 239, but from 294 no reaction was noticed. This organism, much the same as *P. tumefaciens,* has a tendency at times to flocculate in 0.85 salt solution, which may account for the doubtful reactions noted.

The antiserum of *Phytomonas tumefaciens* (Table 9) proved to be specific for its own antigene, except in the case of a 1–40 dilution reaction with *P. vignae* and 1–20 with *P. beticola* No. 1-a. The reaction was very marked in the case of *P. vignae,* and a group relationship between these two organisms may exist. The agglutination with *P. beticola* No. 1-a was very slight, and no strong group agglutination was indicated.

The *Erwinia carotovora* antiserum 395, which was the result of intraperitoneal injections, gave a very low titre. However, it was specific in its action. Except when *Phytomonas tumefaciens* was used as an antigene, there was a definite reaction up to 1–40 dilution. Thus, an apparently contradictory situation was observed, since the antiserum homologous with *P. tumefaciens* in the previous experiment had shown no agglutinative power for *E. carotovora.* Link and Link (6) have previously observed the same phenomenon. It was later found that partial agglutinins for *P. tumefaciens* were actually present in the serum homologous for *E. carotovora.* This was determined by making separate additions of *E. carotovora* and *P. tumefaciens* emulsions, which are concerned in the group agglutination, to aliquots of antiserum. The homologous antigene (*E. carotovora*) when added to the antiserum 395 and incubated for two hours then centrifugalized to obtain a clear serum. This serum was then tested again with the *E. carotovora* and *P. tumefaciens.* No agglutination was obtained with either organism. When the attempt was made to secure absorption, using a suspension of *P. tumefaciens* with another part of the serum in the same dilution, the specific agglutinins for *E. carotovora* were not removed and it was found that the clear serum obtained after incubation and centrifugalization was still capable of agglutinating *E. carotovora.* The clear serum did not agglutinate *P. tumefaciens.* This test is interpreted to indicate that a specific protein is present in *E. carotovora* for the production of

TABLE 10.—*Test of Phytomonas beticola No. 2 and Phytomonas beticola No. 1-a antiserum with various antigenes*

Antigene	Antiserum 295, homologous with *Phytomonas beticola* No. 2							Antiserum 294, homologous with *Phytomonas beticola* No. 1-a						
	Dilutions							Dilutions						
	1/10	1/20	1/40	1/160	1/320	1/640	Check	1/10	1/20	1/40	1/160	1/320	1/640	Check
P. beticola No. 1-a	−	−	−	−	−	−	−	++++	++++	++++	++++	++++	++++	−
P. beticola No. 2-a	++++	++++	++++	++++	++++	++++	−							
P. tumfaciens No. 146	−	−	−	−	−	−	−	±	±	−	−	−	−	−
P. beticola No. 2	++++	++++	++++	++++	++++	++++	−	++	+	+	−	−	−	−
P. beticola No. 1-aa								++++	++++	++++	++++	++++	++++	−
E. atroseptica (1)								−	−	−	−	−	−	−
E. atroseptica (2)								−	−	−	−	−	−	−
E. amylovora								−	−	−	−	−	−	−
Ps. fluorescens								−	−	−	−	−	−	−
E. carotovora								−	−	−	−	−	−	−
P. vignae								−	−	−	−	−	−	−
P. hyacinthi								−	−	−	−	−	−	−

++++ Strong flocculations.
++ Medium flocculation.
+ Slight flocculation.
± Doubtful flocculation.
− No flocculation.

antibodies that will react weakly with *P. tumefaciens,* but the reaction is not reciprocal, *i.e., P. tumefaciens* does not contain antigenic material capable of producing a serum reactive with *E. carotovora.*

The *P. beticola* No. 2 antiserum agglutinated only the homologous antigene and the subculture of serum No. 2, 2-a. No agglutination whatsoever was obtained in cross-agglutination tests in which *P. beticola* No. 1-a or *P. tumefaciens* was employed as an antigene (Table 10).

The antiserum of *P. beticola* No. 1-a from rabbit 294 showed a slight tendency toward agglutination with 1–10 dilution of *P. tumefaciens* and a slight reaction up to 1–40 with strain No. 2. *Phytomonas beticola* 1-aa, which was a reisolation of No. 1-a, when used as an antigene, gave the same agglutination as the parent strain.

The work has shown that sera of relatively high titre are extremely useful for diagnostic purposes if decision is based upon positive agglutinations obtained in high dilutions. *Phytomonas beticola* antisera when tested against other plant pathogenes as antigenes proved to be specific. The negative results of cross-agglutination tests when *P. beticola* strain 2 and 1-a were reciprocally used with the appropriate heterologous antiserum are not unlike results of agglutination tests recorded for strains known to exist within other bacterial species. Strongly positive reactions in high dilutions are apparently significant in showing serological and presumptive specific identity. The negative or weakly positive reactions indicate difference in the antigenes but give little clue to the extent of these differences.

CULTURAL CHARACTERISTICS AND SEROLOGICAL REACTIONS OF R AND S TYPE COLONIES OF PHYTOMONAS BETICOLA

In cultural work with *Phytomonas beticola* No. 1-a there appeared on many plates two distinct types of colonies and in a few rare cases a third type. One was round, regular, yellow, and characterized by a smooth glistening surface; the other more or less irregular, of cheesy consistency, and with a rough or wrinkled surface. Following the terminology of recent bacteriological investigations (*cf.* Hadley 5), the former was termed the S type (smooth), the latter the R type (rough). The third was different from either of the above-mentioned forms and had a rhizoid or lobed outline; and was therefore called the rhizoid type. These three forms have been described by Brown (1) in her recent work and all three forms found to be infectious. In hanging-drop preparations the bacteria from the S types were very motile. The bacteria from the R-type colonies were distinctly more sluggish.

Cultures of either R or S type growing on agar slants remained rather constant to their growth type when regular 24-hour transfers were made.

Dilution plates made from the older (10–20 days) R-type slants also showed considerable constancy in the types present, since only occasionally the S-type colony appeared on the plates (Table 11).

TABLE 11.—*Types of colonies appearing on dilution plates made from 20-day-old R- and S-type cultures. Potato-dextrose agar used in plates 150 x 26 mm.*

a	Parent culture of R type				b	Parent culture of S type			
Plate	R type	S type	Sub-merged	Per cent S type	Plate	R type	S type	Sub-merged	Per cent R type
1-a	6	0	21	0	1-b	10	15	15	40.0
2-a	18	1	12	5.2	2-b	11	9	15	55.0
3-a	10	0	20	0	3-b	10	16	16	38.4
4-a	20	0	9	0	4-b	11	16	8	40.7
5-a	17	0	11	0	5-b	19	19	8	50.0
6-a	19	0	8	0	6-b	6	11	11	35.2
7-a	23	1	8	4.1	7-b	6	16	9	27.2
8-a	42	0	16	0	8-b	23	21	20	52.2
9-a	33	0	15	0	9-b	24	29	12	45.2
10-a	19	2	19	9.5	10-b	7	19	21	26.9
				1.8					41.0

On the other hand, the S-type cultures which had shown constancy-of-growth forms in the 24-hour transfers and which appeared as typical smooth growths, in older tubes gave in a preliminary test, when dilution plates were made from these old tubes, a very large number (approximately 41 per cent) of R-type colonies among the S-type colonies (Table 12).

TABLE 12.—*Number of R- and S-type colonies resulting from selection of the sixth sub-culture of S type of Phytomonas beticola on dilution plates (Transfers made every 24 hours)*

Plate number	Colonies			Remarks
	O–R	S	Submerged	
1	0	2	12	
2	0	13	13	
3	0	10	8	
4	2	19	6	1 R- and 1 O-type[a]
5	0	13	1	
6	1	2	7	O-type[a]
7	0	6	7	
8	0	9	3	
9	0	10	11	
10	0	23	5	

[a] Lobe-type colonies distinct from R- or S-type.

Further work was done to substantiate the above findings; 60 dilution plates were made from many other 20-day-old S-type cultures. The results from these checked well with the first test, since 42.7 ± 4.24 per cent R-type colonies were found to be present among the S-type colonies. It would seem that under conditions present in old culture tubes, the S-type growth form is replaced strongly by the R type and that the R-type growth form is rather permanent.

Certain S-type colonies were selected from the dilution plates and were studied after six transplants to agar slants at 24-hour intervals. All of these cultures appeared to be of the S type in the test-tube cultures. In table 12 the result of a plating from one of these tubes is given. It will be seen that the colonies are prevailingly of the S type, but one R and two rhizoid types appeared.

Very few colonies of rhizoid type have appeared in numerous platings of this organism, the chief colony types being the R and S growths. These are generally clear-cut in their essential features and readily distinguishable. In a few cases there was observed distinct intergradations and these took on some definite aspects. In a number of colonies on the dilution plates made from old cultures of S slants, showing both R and S types, side outcropping of R type was often noticed on S-type colonies. In all instances these outcrops were located near the edge of the smooth colony in a three- or four-day-old plate. A smooth portion was never observed surrounding an entire R-type colony. It was impossible to tell if the two types started to grow in conjunction with each other or whether it was a natural outcrop. Dilutions made from these outcrops always gave a great number of both R and S types.

Attempt was made to differentiate serologically between R and S types of *Phytomonas beticola*. The agglutination and precipitin tests were employed.

The method followed in obtaining a high-titre serum was the same as in the previous discussion on agglutination test. The antigene was obtained by picking out either R or S type from dilution plates. The R antigene when suspended in saline solution had a tendency to clump and settle out, even after three washings. It, however, was used to inject rabbits. The S type remained in suspension without clumping.

The resultant sera from animals injected with the S type showed an average titre of 3600. Sera homologous with the R-type antigene when tested with S antigene gave 1200 titre. It was found that an agglutination test with an R-type antigene was impossible, because of the spontaneous agglutination in various dilutions of salt solution and distilled water. For this reason, if serological differences exist between R and S type they could

not be detected by the agglutination test. It may be pointed out that, judging from the titre of sera obtained with the S type and the R type used as an antigene, the degree of antigenic activity of the S type was much greater than that of the R type. In repeated tests in which the isolations of the S-type and R-type organisms were from the same parent culture, the antisera from the S-injected animals were always much higher in titre than those of the R organisms; the agglutination test being made with the original parent culture as the antigene. A precipitin test was utilized to evaluate the sera homologous with the R type, following the method outlined by Sharp (8). The antigenes were obtained by growing the R and S forms in nutrient broth for 10 days, and the ordinary precipitin procedure was carried out with the clear filtrate (Table 13).

TABLE 13.—*Precipitin test in which broth filtrates of R and S type of Phytomonas beticola were employed against an R-type antiserum*

Type of filtrate	Amounts		Results
	Filtrate	Antiserum	
S	1.0 cc.	1.0 cc.	++++
S	.5 cc.	.5 cc.	++++
S	.25 cc.	.125 cc.	++++
S	.125 cc.	.05 cc.	+
Control[a]	1.0 cc.	1.0 cc.	–
Control[a]	.5 cc.	.5 cc.	–
R	1.0 cc.	1.0 cc.	++++
R	.5 cc.	.5 cc.	++++
R	.25 cc.	.125 cc.	++
R	.125 cc.	.05 cc.	+

[a] Sterile broth filtrate.

The test presented in table 13 shows that the bacterial proteins or proteins that cause the production of the precipitins are so closely related in R and S types that any serological differences could not be differentiated by this test. Similar results were obtained from 6-, 7-, and 8-day-old cultures used as antigenes.

The Pathogenicity and Virulence of R and S Types. The R- and S-type organisms used in plant inoculations were suspensions from 24-hour agar slants, which had been picked from dilution plates of the virulent strain No. 1-aa in the previously described test for constancy of type and which had since been transferred daily.

The sugar-beet plants inoculated were selected from a lot of the commercial variety Pioneer on the U. S. Department of Agriculture Field Lab-

oratory plots at Rocky Ford, Colorado. All beets were in good growing condition and were approximately of the same size. The environmental conditions were uniform and favorable for disease development.

The sugar-beet roots were inoculated near the crown with a hypodermic syringe. A clean sterile syringe and needle were used for each type of inoculum. Irrigation water was kept from the beets for three days, then thorough irrigation was given. The withholding of water was a precaution against washing *Phytomonas beticola* occurring naturally in the soil into the uncalloused wounds of the roots. Irrigation was necessary later to insure the rapid plant growth, so essential to the development of the disease. Readings were made 60 days after inoculations. The actual number of infected beets (Table 14) was practically the same with both types. The

TABLE 14.—*Summary of data on the pathogenicity of R and S types of Phytomonas beticola on commercial sugar beets*

Row No.	Inoculum	Sugar beets			Remarks
		Number used	Galled	Not galled	
1	S	92	60	30	Small galls
2	Check	104	5	95	[a]
3	R	96	65	31	Large galls
4	No. 1-aa (stock culture)	98	8	89	Old cultures
5	" 1-aa " "	25	24	0	24-hr. cultures
6	Check	129	3	118	[b]

[a] Three of these (5) were plow-injured.
[b] Two of these (3) were plow-injured.

results of this experiment showed that the forms are equally pathogenic. If the size of gall is taken as a criterion, then the R forms differ strongly in effect on the host from the S forms. The difference in size of the overgrowths was very marked and, without exception, roots inoculated with the R type showed much larger galls than those inoculated with the S type.

Isolations from the galls arising from inoculations with either the R or S type gave both types on dilution plates, and subsequent inoculations of sugar beets with fresh isolation of the two types from plates made from galls of known origin showed gall formation irrespective of type or origin. No relation of colony type to pathogenicity was found. The findings of other workers on bacterial variations have indicated that the S types are

more virulent, and cases are recorded where the R type is nonpathogenic or attenuated. Sharp (8), working with *Bacterium phaseoli sojense*, has found S types to be the more virulent to soybeans, judgment being based on measurement of spot size. The majority of workers using animal pathogenes have reported the S type as the more virulent. In a few cases exceptions to this situation have been recorded for animal pathogenes. One hypothesis to account for bacterial variation assigns the observed phenomenon to association of characters. For example, Dible (2) has suggested that sorting out of characters takes place in bacteria in different phases of variation, and this results in certain associations such as smoothness and virulence. Exceptions may occur and he recognizes that a character which normally goes with one type of colony may become divorced and be associated with another. Following this suggestion, it would seem at first glance that *Phytomonas beticola* presents one of the exceptional cases. The sugar beet appears to be very susceptible to *P. beticola* and, due to the low resistance, it is possible that it is not a good host plant for determining differences in pathogenicity. The inoculation experiments herein reported show that once infection takes place and the organism becomes established the R type produces the larger galls. If we accept gall size as an index of virulence, the R type could be considered the more virulent. The nature of overgrowth formation in the bacterial-pocket disease probably is one thing and the necrosis in the bacterial-spot disease another. Attempts to produce galls with filtrates have so far given negative results, and there is no evidence to indicate relationship of size of overgrowth to virulence. The fact that the R- and S-type cultures used in inoculation are not exclusively one type, as evidenced by platings both from the culture tubes and galls, makes it impossible to reach a definite conclusion.

The situation as to bacterial variations in *Phytomonas beticola*, so far as is known, is as follows:

Smooth Type (S)	*Rough Type (R)*
1. Colonies on agar plates smooth, glistening, and smooth in outline.	1. Colonies on agar plates rough, cheesy consistency, and often irregular in outline.
2. Suspension stable in physiological salt solution.	2. Agglutination takes place in physiological salt solution.
3. Motility, very marked.	3. Motility, very much reduced.
4. Growth uniformly turbid in broth with slight settling out.	4. Growth forms a heavy sediment in broth.
5. Pathogenic to beets. Overgrowths small.	5. Pathogenic to beets. Overgrowths larger than those from S type.
6. Antigenic properties very strong.	6. Antigenic properties not so strong as in S type.

Previous investigations (Link and Link (6), Sharp (8), Brown (1), and others) have shown the existence of variation in bacterial plant pathogenes which parallels the situation reported for other bacterial species, chiefly animal pathogenes. In the work here reported, the type of colony, whether R or S, was a striking expression of the existence of variation in *Phytomonas beticola*. Morphological and physiological differences were found associated with the difference in colony type. Of these differences, the almost complete absence of motility in the R type may be mentioned. Differences in pathogenicity between the R and S types exist, but conclusion as to the direction of the change cannot be made, since it is not known that the size of overgrowth produced is a measure of virulence. The antigenic properties of the R type are less than those of the S type, but, with present technique, no serological independence can be determined.

It is evident that variation in colony type and in reactions of an organism may occur during the course of a series of tests, and in experimental work this possibility must be recognized.

SUMMARY

1. The bacterial-pocket disease of sugar beets caused by *Phytomonas beticola* has been found in several additional western locations and in a few fields a considerable percentage of the plants were affected.

2. Comparative analyses of diseased beets and of normal beets show that the disease reduces the sugar percentage and the purity greatly.

3. The bacterial-pocket disease has commonly not been distinguished in field work from crown gall and a comparison of the two diseases is given as an aid to ready field diagnosis.

4. The organism enters the host through wounds, injuries from cultivating implements and hail being common entrance points.

5. The organism can overwinter in the overgrowths and as a free-living organism in the soil. In isolation of the organism from the soil, identification of suspects was made by the agglutination test and confirmed by inoculation tests with sugar and garden beets.

6. Serological tests with a number of bacterial species have shown the agglutination reaction to be specific and sera of high titre have been useful in diagnosing suspects, if decision is based upon strongly positive agglutination reactions obtained in high dilutions. Two strains of *Phytomonas beticola* were serologically independent in their agglutination reactions; therefore, negative results of agglutination tests are not to be interpreted as evidence of specific difference.

7. A strain of *Phytomonas beticola* was found to exhibit dissociation which manifested itself by variability in colony type, corresponding to the

rough and smooth types described for other species of bacteria. Subcultures of each type remained rather constant when 24-hour transfers were made, but 20-day-old cultures, originally of the S type, showed approximately 41 per cent R-type colonies in repeated tests. Where the parent culture was of the S type, a very low percentage (2 per cent average) of the colonies from 20-day-old cultures were of the R type.

8. The R-type cultures apparently had fewer antigenic properties than the S-type cultures. Reciprocal agglutination tests could not be made because of the spontaneous clumping of the R-type organisms in salt solution, but the R-type antiserum agglutinated the S-type culture. The R- and S-type antigenes, when used in a precipitin test with an R-type antiserum, gave identical results. Both forms are pathogenic to sugar beets, but the R-type cultures produced the largest overgrowths. It is concluded that *Phytomonas beticola* shows variation comparable to that recorded for other bacteria and this should be taken into account in experimental work.

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TARGET BLOTCH OF SUGAR CANE[1]

C. N. PRIODE[2]

During the past 38 years since Van Breda de Haan (1) described and illustrated what is now known as the eye-spot disease of sugar cane, considerable attention has been given to the leaf spots caused by different species of the Helminthosporium fungi. Until recent years some confusion has surrounded these maladies, because they generally have been considered to be different stages of the same disease.

Van Breda de Haan believed the eye-spot organism to be a species of Cercospora, and named it *C. sacchari.* The descriptions and colored plates published by Lucassen and Went (6) in 1894, by Walker and Went (8)[3] in 1898, and by Krugger (5) in 1899, however, show it to be a species of Helminthosporium and not a Cercospora.

In 1913 Butler and Hafiz (2)[4] reported a leaf spot of sugar cane from India which they called "Helminthosporiose." The fungus causing this disease they named *Helminthosporium sacchari* and it resembled somewhat, in general appearance, the *Cercospora sacchari* described by Van Breda de Haan as the organism causing the eye spot of sugar cane. The color of the spores, the spore measurements, and the descriptions and colored plates of the diseased specimens given by these two authors, however, show it to be a disease separate and distinct from the *C. sacchari* of Van Breda de Haan.

In 1927 Faris (4) described a new leaf spot of sugar cane in Cuba which he called "brown stripe," the name being suggested by the brown, straw-colored spots on the diseased leaves. This disease also is caused by a species of Helminthosporium, and, until the publication of Faris' experiments, was believed to be identical with the sugar-cane eye spot.

During the winter of 1927 what appeared to be an undescribed disease of sugar cane was observed on various plantations in Cuba. Isolation and inoculation experiments proved it to be a new disease caused by Helminthosporium, separate and distinct from any of those mentioned above. It had none of the characteristic symptoms of the eye spot, brown stripe, or Helminthosporiose, and did not readily attack some of the varieties known to be susceptible to these maladies. The incipient stages of the known diseases caused by Helminthosporium on sugar cane are very

[1] Scientific Contributions No. 18, Tropical Plant Research Foundation. From the Cuba Sugar Club Experiment Station, Baraguá, Province of Camagüey, Cuba.

[2] Pathologist, Cuba Sugar Club Experiment Station, Tropical Plant Research Foundation.

[3] See Pl. XXI, Figs. 1–5.

[4] See Pl. I, Fig. 3, and Pl. VI, Figs. 1–8.

much alike in that the infections appear as tiny red specks. This similarity of the early stages of infection is largely responsible for the confusion which has surrounded the identity of the eye spot, brown stripe, and Helminthosporiose. The new disease has this same similarity in its early stages, *i.e.*, infection appears as tiny, red specks where the fungus enters the leaf tissues. As the infection advances, however, characteristic symptoms are produced which readily distinguish it from the other diseases caused by Helminthosporium. Infection usually takes place on the leaf roll, resulting in large, necrotic blotches having irregular, concentric rings which roughly resemble a target board in appearance. Because of this resemblance the disease, in an earlier report by the writer (7), was named "target blotch."

The purpose of this article is to give a more detailed description of the symptoms and to report the results of some further experiments with the disease.

SYMPTOMS OF THE TARGET BLOTCH OF SUGAR CANE

The characteristic symptoms of target blotch are the zonate markings or irregular, concentric rings previously mentioned. These rings are always found on the rolled leaves in the leaf spindle, but the target-board effect is more pronounced after the leaf becomes detached from the leaf roll (Plate I, A). The number of rings produced on an infected leaf varies according to the severity of the infection and the susceptibility of the variety. In some cases only a few rings are formed before the leaf becomes separated from the leaf roll, while in other cases there may be ten or more rings around one infection center. Usually these rings continue to form as long as the leaves remain in a tightly rolled condition in the leaf spindle but cease to develop when the leaf becomes separated from the leaf roll. Infection usually starts with the germination of a spore on the leaf roll. The germ tube enters the leaf tissues, and in a very short time a tiny water-soaked speck may be seen around the point of entrance. As the infection advances the water-soaked area first becomes red, then changes to brown as the spot enlarges. Usually there is very little indication of a halo around the infected areas, but the brown spots soon become surrounded by a mottled, red ring (Plate I, B). As the fungus advances into the healthy tissues this mottled ring becomes necrotic and turns brown, forming the first of a series of brown, straw-colored, concentric rings. As each successive ring is formed by the death of the infected tissues another mottled, red ring is produced by the further advance of the fungus into the surrounding healthy cells. This process of ring development continues until the leaves unfold and separate from the leaf roll. The infection does not appear to spread to any appreciable extent on the unrolled leaves.

The infected area sometimes covers the entire width of the leaf and the target-board appearance is very noticeable (Plate I, A, and Fig. 1).

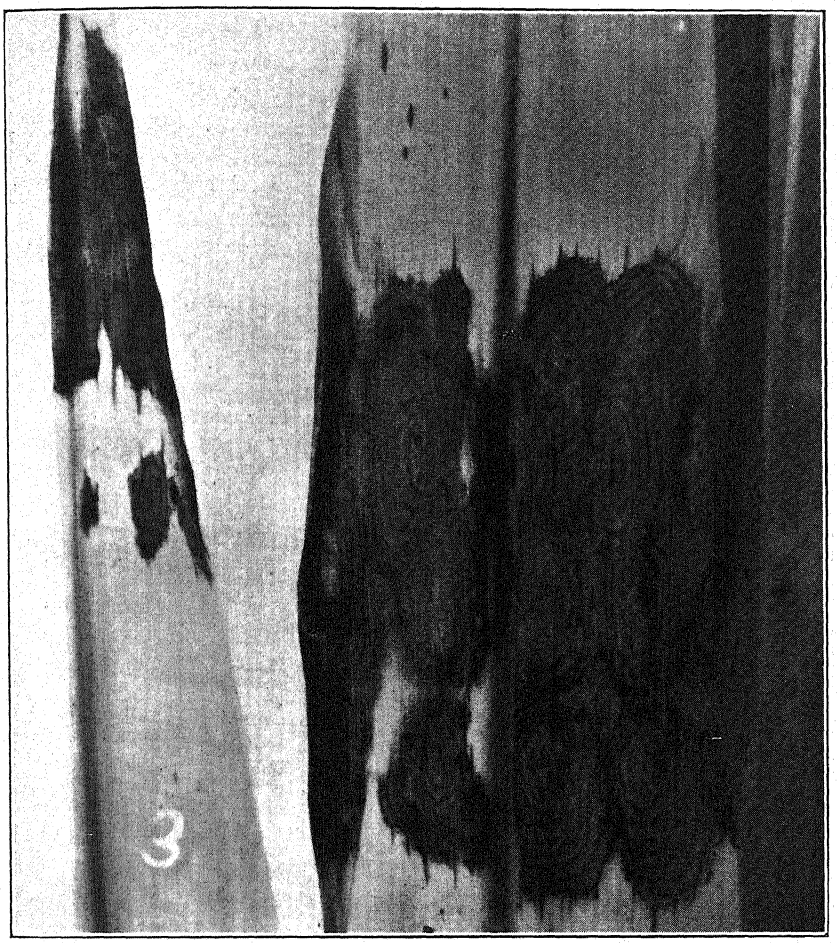


FIG. 1. Infected plant showing typical target blotch on the open leaf. Notice the blotch on the young leaf roll to the left.

The infection on the leaf roll is usually accompanied by a marked depression over the diseased spot caused by the shrinking of the tissues as the diseased cells die (Plate I, B). Infection on the leaf roll usually penetrates the successive layers, forming rings on all the young leaves in the roll. Sometimes the fungus penetrates two or more layers of the same rolled leaf and rings form at each point of penetration. When such a leaf is fully separated from the leaf spindle it appears to have two or more original in-

fection centers, while in reality they all started from the same point of infection. Each spot becomes smaller toward the center of the leaf roll. This is shown very well in Plate I, D. Cases are sometimes seen where several infection centers occur at different heights on the same leaf roll, as shown in plate I, B, and figure 2, A.

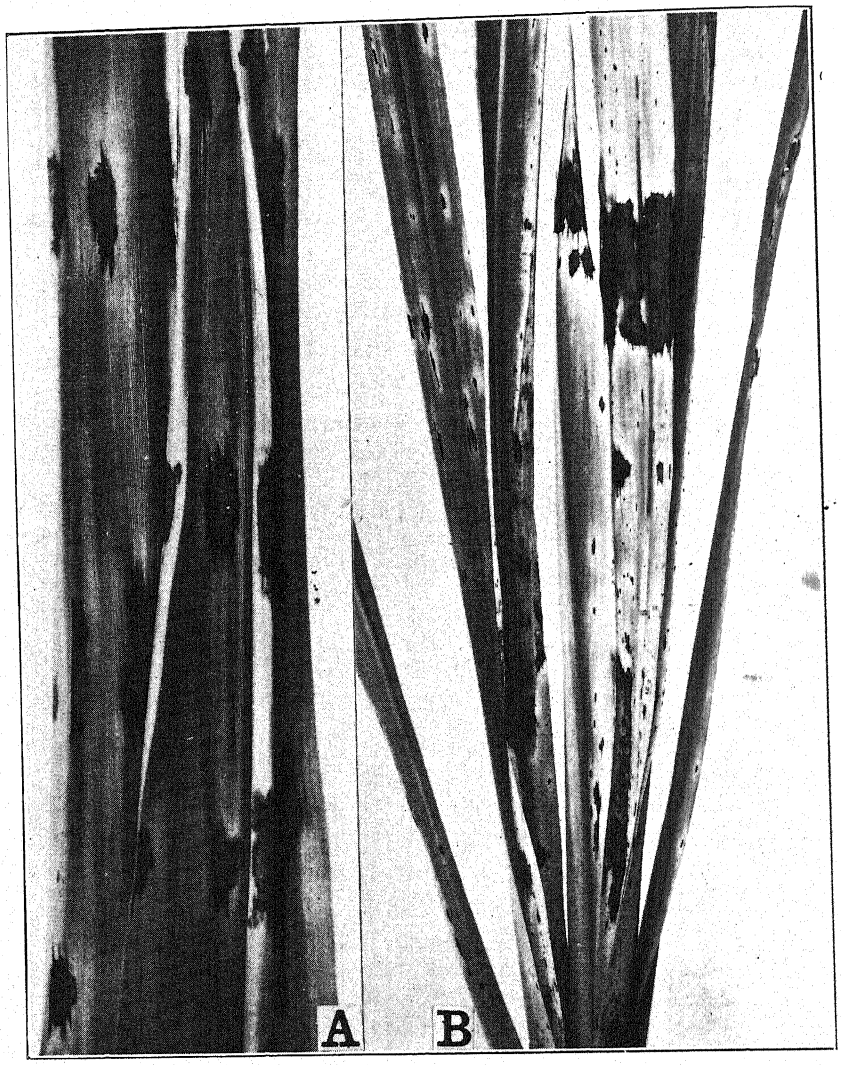


FIG. 2. A. Target-blotch infection occurring at different heights on the same leaf roll. B. Showing numerous small spots or infection centers on the young leaves, most of which resulted from the germination of the spores which were produced on the old blotches above.

On the more susceptible varieties the fungus sometimes penetrates to the center of the leaf roll and extends down to the growing point, causing the death of the whole plant top. Where such cases occur the leaves do not usually unroll but appear to be held together by a web-like mass of fungus mycelium. Spores are produced in large quantities on the old blotches. Numerous tiny red specks or spots can usually be seen on the young leaves below the old blotches. These result, apparently, from the germination of the spores that fall down from the old infections above (Plate I, C, and Fig. 2, B). Sometimes several of these small spots grow together, forming a larger spot or blotch of irregular size and shape. No leaf-sheath or stalk infections have been observed but the fungus has been observed to attack the leaf midrib very readily (Plate I, A). In this respect target blotch differs from both eye spot and brown stripe, neither of which disease attacks the midrib very readily.

ISOLATION OF THE ORGANISM

The first attempts to isolate an organism from plants affected with target blotch were made late in the winter of 1927. The usual method for making isolations was used. Pieces of diseased tissue were surface sterilized, planted in agar plates and incubated at room temperature. In nearly every instance a species of Helminthosporium was obtained which grew very rapidly in agar cultures. Plates planted with diseased pieces in the afternoons usually showed marked growth of fungus mycelium on the following mornings and typical Helminthosporium spores were produced within a short time. Inoculations from these spores on the leaves of vigorously growing plants (no other plants were available at the time) produced only tiny red specks very similar in appearance to the incipient stages of brown stripe and eye spot, but from these infections on older tissues no target blotches developed.

Previous observations having shown that the disease was most severe during the dry cool season and on the more mature canes, further work was suspended until the following December when the disease again made its appearance. At this time isolations were made by the same method as described above and again a Helminthosporium fungus was obtained in nearly every plate. Inoculations made by spraying the spores from some of these plates on the leaves of healthy plants produced typical target blotches, as were observed in the field. Pure cultures were obtained by single-spore isolations and inoculations from these also produced the typical symptoms of the disease. Numerous isolations were made from these infected plants and what appeared to be the same Helminthosporium was obtained in nearly every instance. The fungus is comparatively easy to isolate and grows and fruits very readily in plate cultures.

Pure cultures can nearly always be obtained by unrolling the outer layers of a section of the diseased leaf roll and using the innermost tissues for plating material. Contaminating organisms are seldom encountered when this method is used.

DESCRIPTION OF THE ORGANISM

The general appearance of the target-blotch fungus is not unlike that of the other Helminthosporium species. The color and shape of the conidia

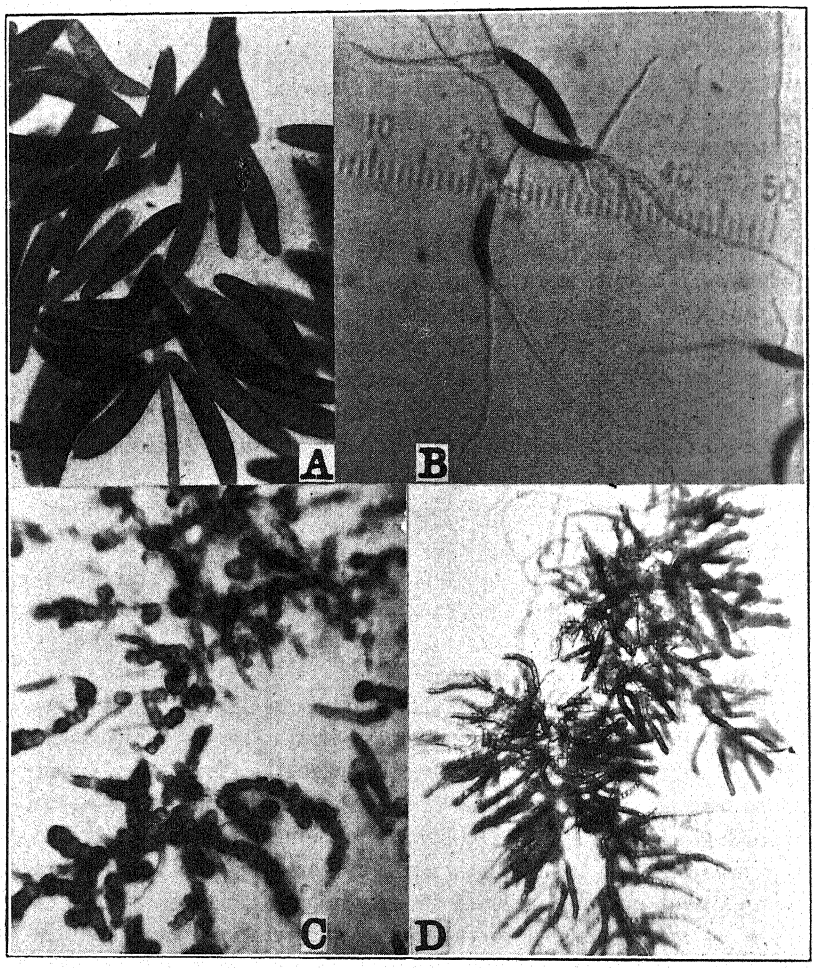


FIG. 3. A. Target-blotch spores from a 5% cane-juice agar culture. ×300. B. Germinating conidia of target blotch. ×112. C. Peculiar bead-like proliferations on the hyphal strands of the target-blotch fungus. Note the size of these growths as compared with the size of the spores. D. Finger-like projections found on the outer edges of certain target-blotch cultures.

on diseased material and on other media are typical of the Helminthosporium group and make easy the determination of the genus (Fig. 3, A). There are certain characteristics of this species, however, which distinguish it from the other species of the group.

The color of the mycelium usually is a whitish gray, but in some cases it may grade into brown or black. The strands which go down into the leaf tissues and the submerged strands in the plate cultures usually are a distinct brown. In petri-dish cultures the fungus makes an irregular, circular type of growth, spreading in all directions from the center. Concentric rings are produced on most media, although they may be almost indiscernible in certain cultures. These rings vary in width and number, according to the media used. On Czapek's agar the growth is very rapid and the rings are wide and few in number, while on 10 per cent cane-juice agar the rate of mycelial growth is rather slow and the rings produced are narrow and more numerous.

The mycelium has a distinctive branching habit, different branches often being connected by short mycelial strands. No swellings or other indications of a zygospore formation have been observed at these unions. Both the aerial and submerged portions of mycelium are numerously septate with varying distances between the septae.

There is also a wide variation in the relative widths of the individual strands, as some are very fine, while others appear coarse and fleshy. As a general rule the submerged portions are larger and more uniform in width than the aerial strands. This, probably, is due to the tendency of the aerial portions to dry out and collapse.

On the outer edge of certain cultures the submerged mycelia sometimes show numerous gnarls or swellings. The ends of the hyphal strands are divided into a great number of short sections by numerous septae. These swellings sometimes present peculiar, though interesting, formations in that the adjoining segments are of unequal shapes and sizes, some being considerably larger than the adjoining ones of the same strand. In some cases these formations consist of short strings of round, unequal-size, bead-like proliferations (Fig. 3, C). In other cases they are made up of finger-like projections of more uniform sizes and shapes (Fig. 3, D). From both of these peculiar types of mycelial proliferations, hyphal strands grow out in much the same way as germ tubes grow out from germinating conidiospores. These strands may arise from the terminal portions, from any intermediate segment, or from both, as is often the case. Within a short time spores typical of Helminthosporium are produced in great quantities on these new hyphal developments.

This peculiar mycelial growth has not been described as a growth characteristic of either the eye-spot or brown-stripe organism, nor is it com-

parable with the mycelial development of *H. giganteum,* described and illustrated by Drechsler (3). So far as the writer can determine, these peculiar growth characteristics have not been described for any other species of Helminthosporium.

There are considerable variations in the responses of the target-blotch fungus to the changes of medium and light. The growth characteristics on the different agars used in these experiments are as follows:

Growth in Czapek's Agar (*Fig. 4, A*). The mycelial growth on this medium is rather profuse and spreads rapidly over the agar surface. The aerial growth is grayish white to brown, while the surface mycelium is dark brown to black. Concentric rings are very pronounced, as shown in figure 4, A. The conidia on this medium are dark brown and usually of a granular appearance. They are rather sparsely produced on the aerial mycelia but very numerous on the surface growth.

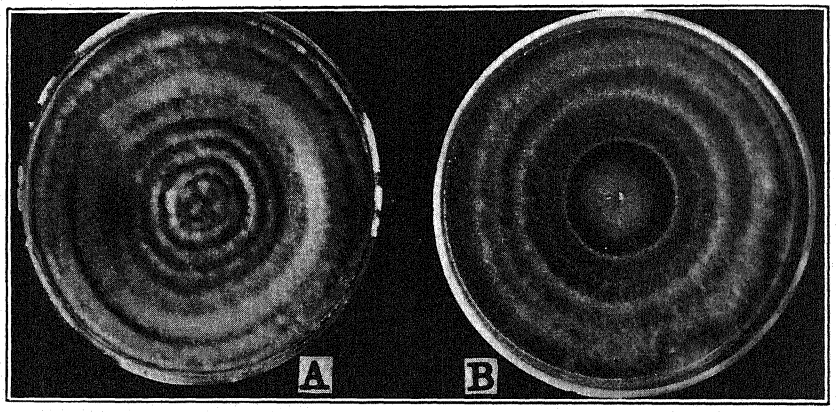


FIG. 4. A. Target-blotch culture on Czapek's agar showing distinct ring formation and considerable aerial growth. B. Culture on cornmeal agar, same age as culture A. The concentric rings are distinct but the aerial growth is not so profuse as on the Czapek's agar.

Growth in Corn-Meal Agar (*Fig. 4, B*). On this medium the mycelial growth is rather sparse but spreads rapidly from the center, forming distinct concentric rings. There usually is very little aerial growth, the mycelium lying close to the agar surface. The color is a light gray to faint greenish brown. The conidia are light brown, produced rather sparsely in center of culture but more abundantly on outer portions, usually forming a black ring around the outer edges of the culture.

Growth in 20% Cane-Juice Agar (*Fig. 5, A*). On this agar the mycelial growth is somewhat thin in center of culture but more profuse on

outer portions. Aerial growth is usually absent in the center but more prominent on the outer two-thirds of the culture. Concentric rings are usually produced, but in most cases are rather indistinct. Conidia are produced scatteringly over whole of culture but are found most abundantly on the surface growth around the outer edges. Both the mycelia and conidia are light brown.

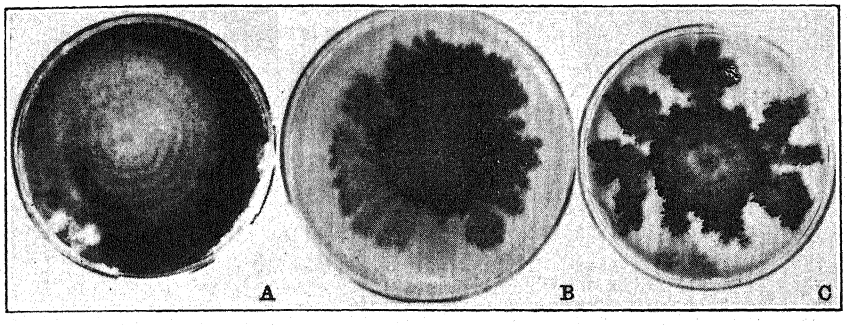


FIG. 5. A. Target-blotch culture on 20% cane-juice agar. Concentric rings discernible in center of culture. Aerial mycelium more profuse on the outer portion of the culture. B. Culture of target-blotch fungus on 10% cane-juice agar. Concentric rings are very faint in center of the culture, but somewhat plainer and more numerous on the outer portions. Outer edges somewhat ragged. C. Target-blotch culture on 5% cane-juice agar. Note the absence of concentric rings and the irregular, ragged appearance of the outer edges of the culture.

Growth in 10% Cane-Juice Agar (*Fig. 5, B*). The mycelial growth on this medium is less profuse than on either corn meal or 20% cane-juice agar. The centers of the cultures usually are thin and lie close to the agar surface. Fringes of aerial mycelium, however, may be seen on the outer portions of cultures. Concentric rings usually are present but in most cases are somewhat indistinct except on the outer portions of the cultures. Conidia are produced abundantly over most of the culture area but are more numerous on the outer portions.

Growth in 5% Cane-Juice Agar (*Fig. 5, C*). The type of growth on this medium differs markedly from that on the other agars. The rate of growth from the center is rather slow and the mycelium lies close to the agar surface. The color of the mycelium is gray to dark brown. Rings are faintly discernible in some cultures and absent in others. The mycelium grows out from the center in all directions and forms a dark brown to densely black ring around the outer edge. This ring is caused by the production of numerous conidia on the peculiar hyphal proliferations previously described. From different points on the outer edges of this black ring, single mycelial strands grow out a short way and begin to branch from

a certain point or from several points along their sides. As these little branching points or growth centers continue to spread, they appear to the unaided eye as little individual growths or cultures. Under the microscope, however, the single mycelial strand connecting them to the body of the main culture can be seen very plainly. The mycelial growth of these little centers is usually made up of gnarly proliferations similar to those observed around the edges of the main culture. As the mycelial growth continues to spread, these little growth centers are soon connected to the other part of the culture. From the outer edges of these new developments other strands grow out and start new growths. This method of spread gives the cultures a ragged and peculiar appearance, not observed on the other media used in these experiments. The type of growth is shown very plainly in figure 5, C. In a few cases the outer edges of cultures growing in 10% cane-juice agar approached this type of growth but this characteristic was not usually so pronounced as in the 5% cane-juice agar (Fig. 5, B).

The influence of light on the growth of the target-blotch fungus is very pronounced. Figure 6 shows two single-spore isolations of the same age.

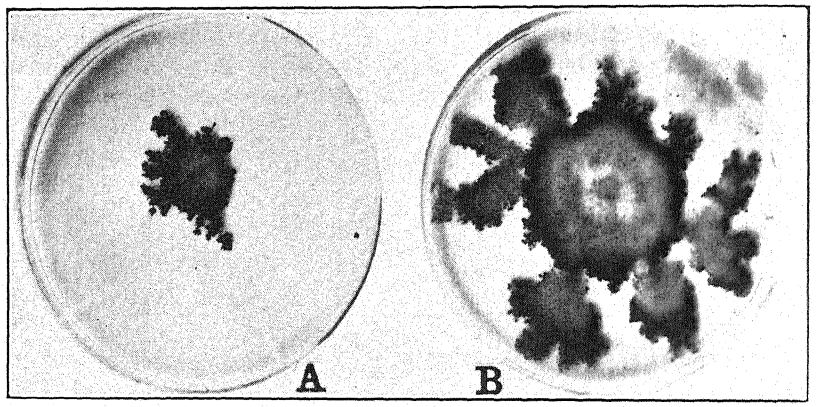


FIG. 6. Two single-spore isolations of the target-blotch fungus. These cultures were plated at the same time and on the same class of agar (5% cane juice). A. Grown in the photographic dark room from which all daylight was excluded. B. Grown in the ordinary daylight of the laboratory.

These were taken from the same culture, plated on the same class of agar (5% cane-juice agar) and grown under the same conditions except that culture A was kept in the photographic dark room from which all daylight was excluded, while culture B was grown in the ordinary daylight of the laboratory. It will be noted from this illustration that the exclusion of light greatly retarded the mycelial development, the size of the culture

grown in the dark being much less than that of the one grown in the light. The production of conidia, however, appears to be stimulated by the absence of light. Spores are produced in innumerable quantities over the whole of culture A, while in B they are most abundant on the black portions around the edges of the culture. Cultures on other classes of media treated in the same way showed a marked difference in the type of resultant growth.

The conidia on the different media are very similar, differing slightly in size and density of color. They are typically light brown to dark brown, depending on the medium on which they are grown. Spores taken from diseased cane leaves usually are light brown.

Microscopic examinations of spores from target-blotch, eye-spot, and brown-stripe cultures show the target-blotch conidia to have a color somewhat darker than the eye-spot spores but considerably lighter than the conidia from the brown-stripe cultures.

The shape of the target-blotch spores is typically slightly curved, the curve being more pronounced on one side due to the bulge of the spore. The thickest part usually is toward one end rather than in the center of the spore (Fig. 3, A).

Germination is typically from the polar segments. The germ tubes usually begin branching very close to the end of the spores (Fig. 3, B). No instance has been observed where germination took place from the intermediate segments. The germination of the spore and growth of the mycelium are very rapid. Under favorable conditions target-blotch spores will begin to germinate within two hours after they have been plated and after twelve hours will have made considerable mycelial growth.

The results from several hundred spore measurements show the average size of the target-blotch spores to be 15.4 $\mu \times 76.2\ \mu$. According to Faris (4) the average measurements of the eye-spot and brown-stripe spores are 12.7 $\mu \times 69\ \mu$ and 17 $\mu \times 84\ \mu$, respectively. A comparison of these measurements shows that the target-blotch spores are larger than the eye-spot but smaller than the brown-stripe conidia.

INOCULATION EXPERIMENTS

The first inoculations from isolated material, as stated elsewhere in this report, were made on vigorously growing, young canes. These inoculations were made by spraying a suspension of the spores in distilled water on leaves of the young plants with a small atomizer. After about two days numerous small red specks were observed on the sprayed leaves. There were no indications of the characteristic rings or blotches of the disease. Results from later experiments suggested this to be due to the rapid growth

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of the young canes and to the high temperatures, factors which are unfavorable to the development of the fungus. No further work was done until December, 1928, when new inoculation experiments were started. Several plants of the C 760 variety were inoculated with spores from cultures of single-spore isolation. About twenty-four hours later numerous small red specks appeared on the unrolled leaves. Some of these spots grew together, forming larger spots or blotches but no target blotches developed. On the spindle rolls the infection appeared as small specks, similar to those on the unrolled leaves, but in a short time these small centers of infection became larger and the affected tissues somewhat sunken. Mottled red rings developed around the spots and, as the infection advanced, these red mottled zones became necrotic and changed to a brown straw-color, forming the concentric or zonate rings previously described. On these plants, as on the infected plants observed in the field, the rings continued to form until the leaves became separated from the leaf roll. In most cases the fungus penetrated to the center of the leaf roll and rings were formed on all the leaves present in the spindle roll at the time.

The fungus was reisolated from the infected plants and grown in pure cultures. Spores from these cultures were sprayed on the leaves of several plants of the following varieties: C 47; C 49; C 69; C 760; PR 560; PR 551; SC 12/4; Cristalina; FC 306; D 109; BH 10/12; and Badila. Other plants of these same varieties sprayed with distilled water alone served as checks. After about two weeks target blotches were showing on all of these varieties except FC 306 and D 109. The checks remained healthy. The Cuban and Porto Rican seedlings appeared to be most susceptible. On these varieties the typical target-blotch symptoms developed very readily, but only on the leaf rolls. The results from these and former experiments seemed to indicate that the concentric rings were formed only when infection took place on the leaf roll. To determine this, inoculations were made as follows: Several plants of the C 760 variety were inoculated by spraying the spores on the open leaves only, care being taken to prevent any of the inoculum falling on the leaf rolls. Several other plants of the same variety were thoroughly sprayed on both the open leaves and the leaf rolls. Within a short time the incipient stages of the disease could be seen on all the inoculated leaves of both sets of plants, but the uninoculated spindles of the first set remained free of infection. About ten days later typical target blotches developed on the inoculated spindles of the second set of plants but the spindles of the first set continued free of the disease. No rings formed from any of the inoculations on the unrolled leaves. The results from these and similar experiments performed later show definitely that the rings develop only when infection takes place on the leaf roll. The fungus apparently cannot spread in the tissues of the open leaves.

A mass of fungus mycelium can usually be seen between the layers of leaves in the infected leaf rolls and the spread of infection in the leaf rolls with the subsequent formation of concentric rings appears to be due to the spread of the mycelial hyphae between the leaf surfaces rather than to the advance of the fungus within the leaf tissues. This spread of infection apparently takes place as follows: the mycelial growth develops around the

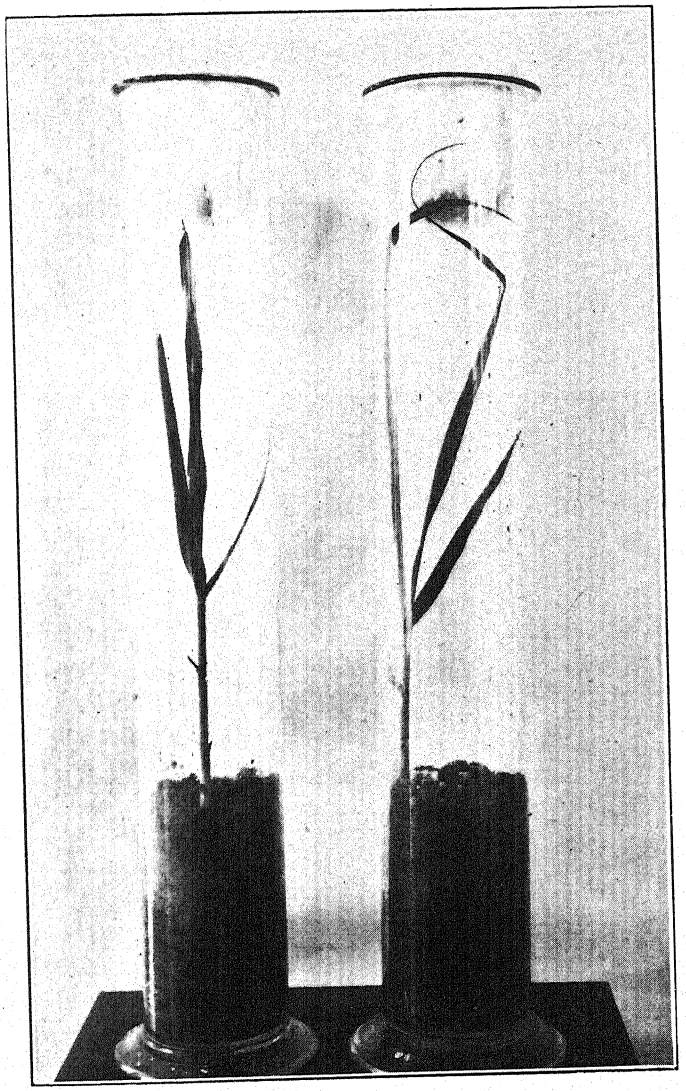


FIG. 7. Two young cane plants infected with target blotch. Showing method of growing and inoculating plants under strictly aseptic conditions.

edges of the diseased spots between the leaf surfaces and sends haustoria into the healthy cells. As these cells become infected, a mottled red ring is formed which later becomes necrotic and brown. The hyphae of the mycelium then spread across this necrotic ring and send haustoria into the surrounding healthy cells with the subsequent formation of another brown ring. This process of ring development continues as long as the leaves remain in the leaf spindle, but ceases when the leaves unroll due to the drying out and collapsing of the mycelial strands on becoming exposed.

In order to obtain infection under more uniform conditions inoculations were made on plants grown in tall glass cylinders or jars under strictly aseptic conditions. This method of growing and inoculating plants was worked out by Faris (4) and used in his experiments with the eye-spot and brown-stripe diseases. The method is described as follows: the tall glass cylinders were filled to a height of three or four inches with clean sand moistened with a culture solution suitable for the growth of the cane. The jars were stoppered with cotton plugs, the tops wrapped with wrapping paper, and the whole sterilized in a large autoclave at 25 lbs. steam pressure for five hours. The jars were then brought to the laboratory and planted with single-eye seed pieces of the variety C 760, the seed pieces having first been sterilized for 15 minutes in a 1:1000 solution of mercuric chloride. They were covered with sand from smaller cylinders, sterilized along with the larger cylinders. When the cane germinated and had reached a height of six to eight inches they were sprayed with a spore suspension from a single-spore isolation of the fungus. In a short time the leaves of the young plants were thickly covered with small red spots and typical target blotches soon developed on several of the young leaf rolls. Two of the jars with the infected plants are shown in figure 7. The infections produced under these aseptic conditions were free from contaminations of any kind and proved definitely that target blotch is caused by the Helminthosporium fungus.

DISTRIBUTION AND SEASONAL OCCURRENCE

Target blotch was first observed in 1927 on the experiment plots at Central Baraguá. Since that time it has been observed at Central Pilar, Central Jatibonico, Central Jagueyal, and Central Carmita, and probably occurs elsewhere in the island. During the fall and winter of 1927 and 1928 it was found to be rather prevalent on most of the varieties at this station. Some of these varieties were severely attacked, while others were only lightly infected. Usually the spots or blotches are found in the locality of Central Baraguá about the 10th to 15th of December. As the dry, cool weather continues the attacks of the fungus become more severe and widespread. The cool, dry weather and the slow-growing canes at this time

of the year seem to favor the development and spread of the disease. With the coming of the warm, wet weather in the spring the disease gradually disappears except, perhaps, where the old blotches from earlier infections may still be seen.

VARIETAL SUSCEPTIBILITY TO TARGET BLOTCH

Observations during the past two years show that very few of the varieties in the varietal test plots at this station are immune from target blotch. Some of these varieties are highly resistant and show very little effect from the disease, while others are rather severely attacked. On the resistant varieties only a few target blotches may be seen, infection for the most part showing as very small red spots on the unrolled leaves. The susceptible varieties, however, usually show numerous blotches and in a great many cases the tops are killed. Certain of the Cuban and Porto Rican seedling canes are most susceptible but fortunately these canes are not now being grown commercially in Cuba. The newly-introduced POJ varieties, POJ 2883, POJ 2878, POJ 2727, POJ 2725, POJ 2722, POJ 2714, and POJ 36, and CO 281, CO 213, and H 109 are highly resistant to the disease. No typical target blotches have been observed on any of these varieties. Cristalina, Santa Cruz 12/4, and BH 10/12 become infected very readily but usually with slight resulting damage.

The following table gives the relative susceptibility of cane varieties to target blotch as observed on the varietal test plots at this station during the past two winters.

It will be noted from the above table that a great many varieties of sugar cane are susceptible to the attacks of the target-blotch fungus. This table, however, is by no means complete, for there are many varieties which we have had no opportunity to observe in connection with the disease. Only those susceptible varieties, now being grown on the variety test plots at this station, are listed in the above table. Several of the varieties which now appear to be highly resistant to the disease might, under varying conditions, prove more susceptible if observed over a longer period of time. The losses from target blotch on these susceptible varieties usually are small due to the fact that the conditions most favorable for the development and attacks of the fungus do not develop until the canes are well on toward maturity. However, under favorable conditions and on new and perhaps more susceptible varieties, that find their way into the island from time to time, the disease might prove to be more destructive. It therefore is very important that all new varieties be rigidly tested for their susceptibility to this and other diseases before they are distributed as field canes.

TABLE 1.—*Relative susceptibility of cane varieties to target blotch*

1. Varieties severely attacked and on which several typical target blotches usually occur. Infection sometimes results in the death of the entire leaf roll.

C 760	C 145	C 28	PR 724	PR 545
C 228	C 33	C 14	PR 551	HE 45

2. Varieties on which several blotches were observed but whose tops usually were not killed.

C 350	C 31	PR 561	SC 12/4
C 251	C 30	PR 540	BH 10/12
C 69	PR 719	PR 492	B 1809
C 40	PR 700	Cristalina	Yellow Bamboo

3. Varieties on which only a few cases of infection were observed.

C 45	C 24	C 14	PR 449
C 34	C 23	PR 543	PR 417
C 27	C 20	PR 460	PR 260

4. Varieties on which infection is usually very mild. Only a few typical blotches observed.

C 588	C 67	C 47	PR 707	B 67
C 576	C 63	C 46	PR 704	HVR 6307
C 502	C 62	C 45	PR 701	HVR 6159
C 491	C 61	C 44	PR 700	HVR 5039
C 483	C 60	C 43	PR 443	HVR 4124
C 338	C 59	C 42	PR 409	Badila
C 291	C 56	C 17	PR 329	Negrita
C 220	C 55	PR 724	PR 260	Yel. Caledonia
C 145	C 54	PR 719	PR 209	Baraguá 2
D 74	C 50	PR 712	B 3412	BSF 1248
D 108	C 49	PR 709	B 1753	
			B 306	

SUMMARY

1. The symptoms of target blotch are described and illustrated.
2. Isolation and inoculation experiments proved the causal organism to be a species of Helminthosporium. The identity of the species, however, has not as yet been determined.
3. The growth characteristics of the fungus in plate cultures are described and illustrated. The mycelial development on certain media presents some very interesting studies.
4. The attacks of the fungus are most severe on the more mature canes during the winter season.
5. Many varieties of cane are shown to be susceptible to the attacks of the fungus.

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EXPLANATION OF PLATE I

A. A typical target blotch on sugar-cane leaf showing numerous concentric rings.

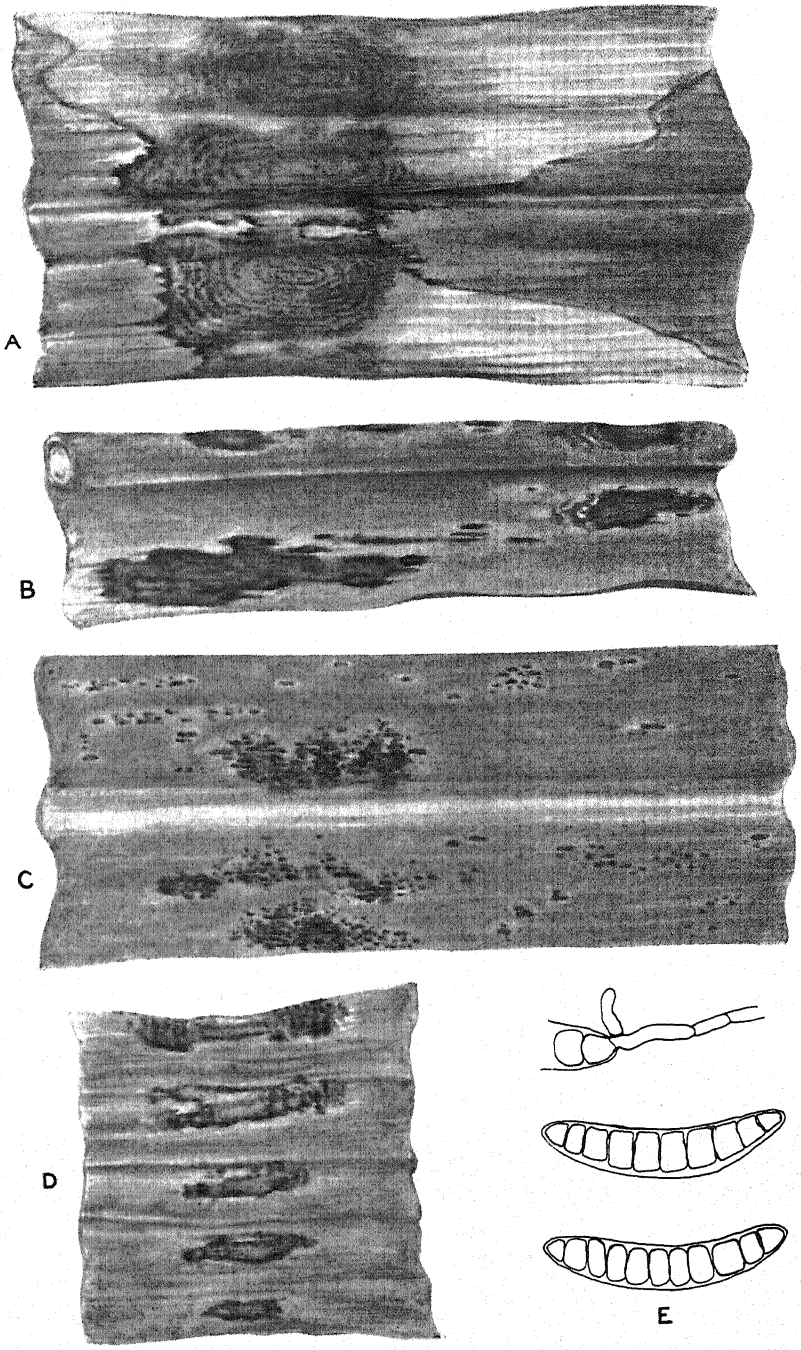
B. Early stages of target-blotch infection on a leaf roll of sugar cane showing the mottled red rings surrounding the diseased spots and the shrunken condition of the infected tissues.

C. Small infection centers of target blotch on an unrolled leaf of sugar cane. These little infection centers do not enlarge on the unrolled leaves.

D. A section of a young leaf of sugar cane unrolled to show the penetration of the fungus through the successive layers of the rolled leaf.

E. Inked-in photographs of target-blotch spores, × 400.

L.C.C. Krieger print.



SUGAR-BEET YELLOWS CAUSED BY FUSARIUM CONGLUTINANS VAR. BETAE

DEWEY STEWART

An unreported disease of sugar-beet roots has been found to occur in Colorado, especially in the southeastern portion, commonly known as the Arkansas Valley. The disease may have been overlooked or previously assumed to be a peculiar manifestation of root rot due to one of the organisms known to be involved in that disease complex. The symptoms of this disease are very distinct, however, and should not have been confused with other diseases of beets. Furthermore, the causal organism as well as the symptoms of the disease has not been described, so it seems probable that a new disease of sugar beets has appeared whose present and potential importance warrant a report.

The name yellows, which fairly well describes the striking appearance of the diseased plants and which has been used for similar diseases of cabbage and celery, is applied to this disease.

SYMPTOMS

Leaf Symptoms. The most characteristic leaf symptom is a yellowing of the mature leaves which gradually appears on the younger ones (Fig. 1). As the disease progresses the heart leaves generally show distortions in the form of an inrolling of the edges and a twisting of the apex to one side (Fig. 2). Leaves showing these distortions lose their pliable texture and appear brittle when grasped in one's hand. Wilting apparently does not occur in large plants under natural conditions; however, wilting frequently occurs with little or no yellowing of the leaves when greenhouse plants are inoculated.

The first symptom presented by large leaves is a yellowing of the portion of the leaf blade between the large veins. Within a few days the entire leaf takes on a yellowish to gray color, except the large veins and a narrow border of leaf mesophyll which may remain green. Shortly, the large veins and petioles die and the dead or dying leaves become heaped around the crown of the diseased plant (Fig. 3).

Root Symptoms. Roots of plants with yellows present almost no external indications of a disease; however, if the root is cut across, the grayish to brown discoloration and rot of the vascular system are evident. The vascular tissue of beet roots occurs in concentric rings (Fig. 1). In most cases only a portion, consisting of one or more rings, of the vascular tissue on one side of the root is involved, which explains the frequent occurrence



FIG. 1. Sugar beet affected with yellows caused by *Fusarium conglutinans* var. *betae*. Early stage showing color change in the larger leaves accompanying the vascular invasion and dry rot of the root. Insert B shows a portion of diseased root in which the vascular involvement is pronounced.

of individuals whose leaves on one side show yellowing and, even, distortions, while leaves on the other appear healthy. Eventually all the leaves of an affected plant show symptoms of disease, as the organism spreads laterally, involving other parts of the root.

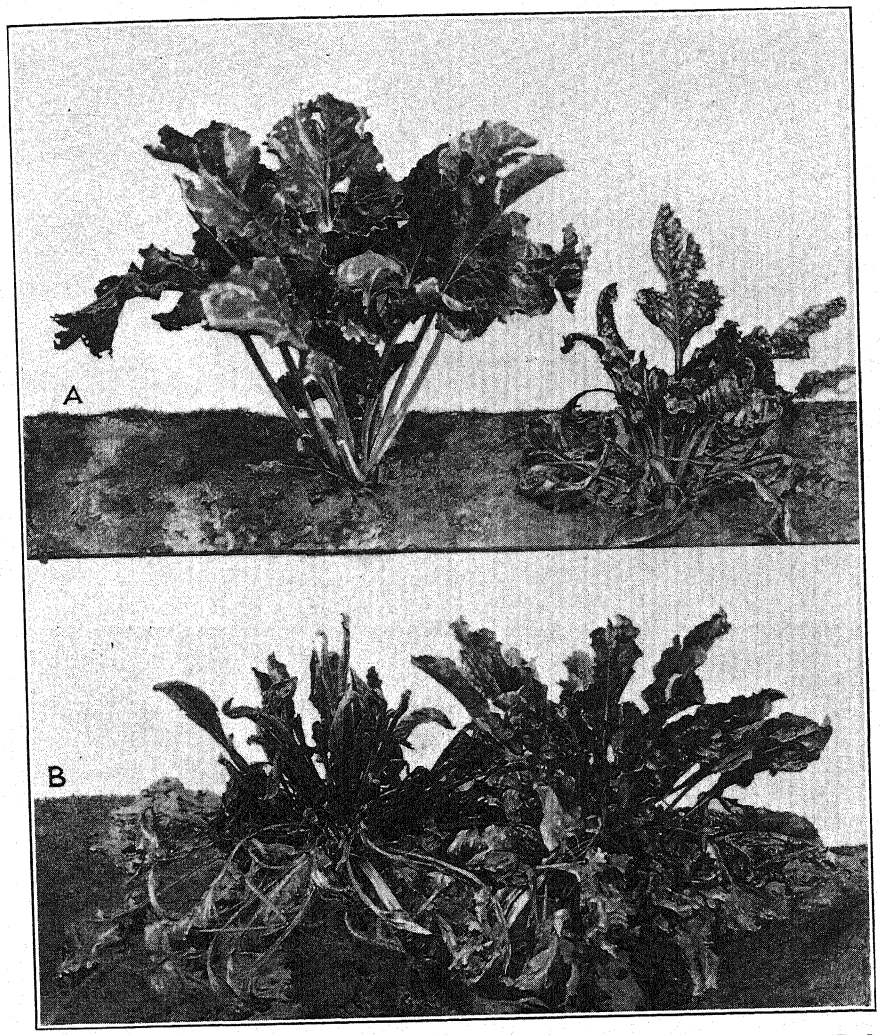


FIG. 2. Normal sugar beets contrasted with those affected with yellows: A, Early-season aspect (diseased plant at right). B, Late-season aspect of the disease showing the diseased plant (left) with only one or two of the mature leaves alive and the heart leaves furled and distorted.

By gradually cutting away a diseased root from crown to tip the path of the fungus invasion can often be traced to the starting-point in a lateral root, usually located 5–8 inches below the surface of the ground. It would seem that the pathogene gains entrance through small lateral roots rather than wounds, and the occurrence of the initial infection at certain depths is thought to be related to the prevalence of lateral roots rather than to a restricted location of the fungus in the soil.

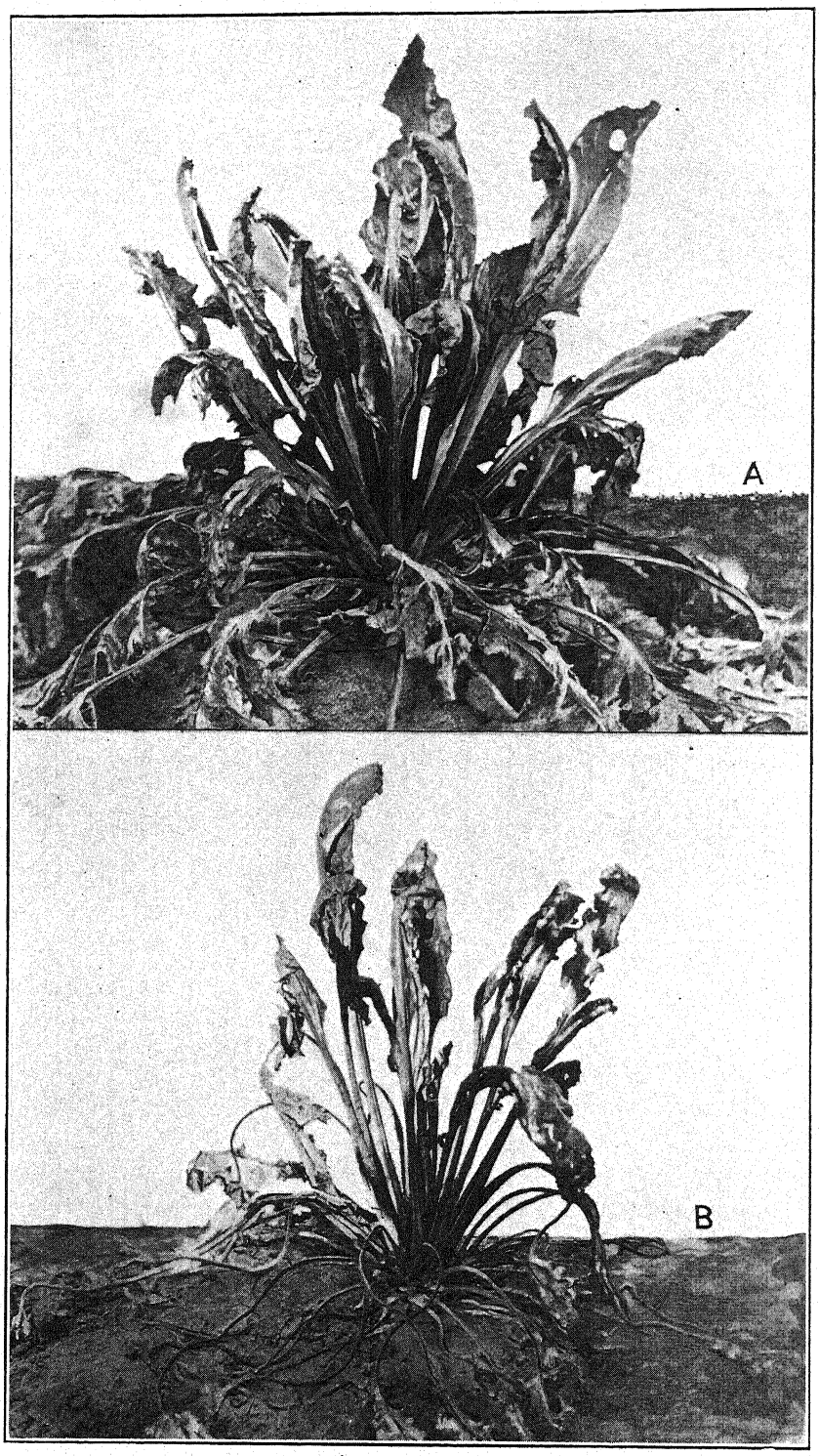


FIG. 3. Sugar beets in late stages of yellows: A, Older leaves dead and the leaves of the inner whorls distorted. B, Older leaves dead, growth of heart leaves completely checked.

Seedling Symptoms. The symptoms manifested by seedlings with yellows may be a typical wilt in which the leaves and cotyledons become flaccid, curled, and dry without ever showing a yellow color. In larger seedlings the leaves may become yellow, duplicating the leaf symptoms of large plants in the field. In most cases after the cotyledons and leaves are diseased the stem remains upright with no apparent injury; however, if the stem is broken the vascular system shows a grayish to brown color. The tendency for the diseased seedling to remain upright, as well as the general absence of external decay, usually distinguishes this disease from ordinary damping off.

IMPORTANCE

The value of beet roots is determined by the weight and the percentage of sugar they contain. Beets low in sugar are discriminated against, due to problems they present during the processes of beet-sugar fabrication, and some companies reserve the right to reject beets testing less than 12 per cent sucrose. This disease has been found to produce a marked effect on the sugar percentage and a depressing effect on the weight of roots.

Analyses were made of healthy and diseased roots in order to determine the influence of this disease upon weight and percentage of sucrose. At the time of collecting the diseased plants, the healthy one growing next along the row, which was produced under similar conditions except for disease, was taken for comparison. Table 1 gives in summary form the results of the determinations from 15 beets of each class.

From these data it is evident that the percentage of sucrose is reduced in the diseased plants even from the first indication of disease. The greater reduction in percentage of sugar is found in the individuals showing the more advanced stages of disease. The diseased roots, judging from the determinations at harvest, show a significant reduction in weight.

Since sugar beets are susceptible to the pathogene from seedling stage to maturity a loss of plants occurs throughout the season in infested fields. The total loss of individuals in a particular field due to this disease has not been determined, yet as many as 1 per cent of the plants have been found diseased at the time of making a survey. Probably more important than the present-day losses is the fact that beet yellows belongs to that increasing group of plant diseases having as their causal agent some species of the genus Fusarium, all of which are very difficult to control.

ETIOLOGY[1]

The fungus causing beet yellows belongs to the genus Fusarium; however, complete sporulation, which would enable one to compare it with

[1] The etiological studies were made while the writer was a graduate student at Cornell University and he wishes to express his appreciation for the facilities of the Department of Plant Pathology.

TABLE 1.—*Comparison of roots from yellowed and healthy plants. Plants collected near Rocky Ford, Colorado—1927. Results given as averages of 15 individual readings*

Time of collecting	Weight (gms.)			Percentage of sugar		
	Healthy	Diseased	Difference	Healthy	Diseased	Difference
Late summer						
Slight disease	552.0 ± 39.16	447.0 ± 22.12	105 ± 47.15	10.36 ± .60	8.20 ± .53	2.16 ± .80
Severe disease	535.5 ± 70.88	418.5 ± 56.50	117 ± 90.64	10.68 ± .39	6.68 ± .29	4.0 ± .48
Just before harvest						
Average	895.9 ± 37.93	353.0 ± 39.59	542.0 ± 54.8	12.00 ± .27	7.67 ± .37	4.33 ± .46

known species of the genus or establish a new species, has not been obtained. The fungus resembles *Fusarium conglutinans* Wollw. more than other Fusaria and is provisionally placed as a variety of this species. On the basis of its morphological and physiological characters the organism has been named *F. conglutinans* var. *betae*.

Isolation of the organism from affected tissue can readily be accomplished, but, since a sugar-beet root offers an excellent substratum to most soil fungi, after its natural resistance is once broken down, precaution must be taken to make sure that only recently invaded tissue is used. In order to make more certain of having the proper disease, when first attempting to isolate the pathogene, only plants showing marked symptoms were used. This material for the most part gave cultures of Fusaria. The prevailing type of culture was characterized by a peculiar cottony white mycelium and its failure to produce a pigment on glucose agar. In a few isolations the Fusarium obtained produced various shades of red pigment. These undoubtedly were secondary invaders, since later inoculations failed to produce disease.

The association of this particular organism was further demonstrated when isolations were made from plants showing the first symptoms of disease. The bit of tissue plated was always taken from the region separating diseased and healthy portions of the root. The results of isolations made from 103 plants in this manner are given in table 2.

TABLE 2.—*Organisms isolated from roots of sugar beets with yellows*

Location	Date 1927	No. roots	No. roots with *Fusarium conglutinans* var. *betae*	Other organisms
Manzanola, Colorado	June 26	7	7	0
Rocky Ford, Colorado	July 8	50	33	5 bacteria 12 no growth
Rocky Ford, Colorado	Aug. 12	21	20	1 Rhizoctonia
Rocky Ford, Colorado	Aug. 23	25	23	2 no growth
	Total.....	103	83	

Thus, from 103 plants with yellows, there was found associated a particular type of Fusarium in 80 per cent of the roots. If the instances of "no growth" are accounted for as excessive sterilization of the piece of tissue and the bacterial growths as contaminations, there remains only one failure to secure *Fusarium conglutinans* var. *betae* from plants in the early

stages of this disease. Since species of Rhizoctonia are commonly associated with root rots of sugar beets the one isolated is undoubtedly a secondary pathogene or the early symptoms of yellows may have been confused with the disease produced by this Rhizoctonia.

The pathogenicity of *F. conglutinans* var. *betae* was demonstrated by the following method. Healthy sugar-beet plants about 6 weeks old growing in small pots were transferred, with the adhering dirt, to a larger pot of sterilized soil. Some of the pots were inoculated with *F. conglutinans* var. *betae* and within three weeks typical symptoms of beet yellows appeared, while the plants transferred to soil without the organism remained healthy. Tissue platings were made from the small lateral roots as well as the vascular tissue of the main root of these diseased plants and in every case a Fusarium similar to *F. conglutinans* var. *betae* was obtained.

CLASSIFICATION

The Fusarium causing sugar-beet yellows has been grown on many kinds of cultural media, such as stems of sweet clover, red clover, alfalfa, tomato, and alnus, beet petioles, beet and potato plugs, as well as synthetic and vegetable-decoction agars, at temperatures varying from 3° to 33° C. and hydrogen-ion concentration varying from pH 3.7 to pH 9.2, but in no case have macroconidia been produced. The outstanding cultural character of the organism is the absence of pigment on steamed rice, which is characteristic of relatively few species of Fusaria. Wollenweber[1] used the absence of pigment on steamed rice as a basis for separating *Fusarium conglutinans* Wollw. from *F. orthoceras* Appel and Wollw. *F. orthoceras* is a soil saprophyte or a weak parasite, while the orthoceras-conglutinans group contains important pathogenes, such as the aster-wilt[2] organism and the pea-wilt organism recently reported by Linford.[3]

Cultures of the organism causing beet yellows were submitted to C. D. Sherbakoff but, he being unable to obtain macroconidia, stated "that superficially it resembles *F. conglutinans* more than other Fusaria." He pointed out, however, "certain peculiarities by which it apparently differs from *F. conglutinans* or *F. conglutinans* var. *callistephi;* namely, it produces extremely fine ultimate hyphae which often grow in rather characteristic curves— also differs from them in the chlamydospores which in your Fusarium are often observed in fairly long intercalary chains."

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[2] Beach, W. S. The Fusarium wilt of China aster. Rpt. Mich. Acad. Sci. 20: 281–308. 1918.

[3] Linford, M. B. A Fusarium wilt of peas in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 85. 1928.

Cultures of *Fusarium conglutinans* and *F. conglutinans* var. *callistephi* were secured from G. H. Coons, formerly of the Michigan State College, as well as J. C. Gilman, Iowa State College. These Fusaria along with the one from sugar beet were used to inoculate pots of sterilized soil. The pots were inoculated with a single organism and then equal portions planted to sugar beets, cabbage, and asters. In every case disease occurred only in the species of plants from which the organism was originally secured. The inability of the sugar-beet Fusarium to produce disease in cabbage or aster was further demonstrated by inserting the organism into stems of plants, and no indication of a disease resulted. Likewise, beet roots were inoculated with *F. conglutinans* and *F. conglutinans* var. *callistephi* without the production of disease symptoms.

These tests show that *F. conglutinans* var. *betae* is distinct from *F. conglutinans* or *F. conglutinans* var. *callistephi* in pathogenicity.

Technical Description.—**Fusarium conglutinans** var. **betae**, nov. var.

The microconidia are hyaline or grayish in mass, 2–3 μ wide and 7–12 μ long; continuous except for an occasional septate spore in old cultures; straight to slightly curved. The chlamydospores are globose to ovoid and may be terminal or intercalary, the latter are generally produced in long chains. The mycelium is cottony white with aerial growth abundant. Its most striking cultural characteristic is the absence of pigment production on steamed rice. Pathogenic to sugar beets, producing disease called yellows. Found in commercial beet fields in Colorado.

PHYSIOLOGY

Growth on Media. The organism will grow on almost any vegetative substrate or agar of common formula; however, the abundance of mycelium produced depends upon the amount of soluble carbohydrates present.

Temperature—pH. The influence of temperature and reaction of the medium on the growth of *Fusarium conglutinans* var. *betae* were deter-

TABLE 3.—*Growth of Fusarium conglutinans var. betae agar at different pH and temperature*

	Temperature °C.										
	3	6	9	12	15	18	21	24	27	30	33
pH	Growth in cm.										
3.7	0	0	+	0.9	1.2	2.7	3.1	3.9	3.9	2.0	1.0
4.7	0	0	+	1.4	2.2	3.5	4.2	5.6	5.7	3.2	2.0
5.8	0	0	+	1.8	2.7	4.2	4.8	5.8	5.9	3.6	2.5
7.0	0	0	+	1.5	2.4	3.5	4.5	5.5	5.5	2.7	1.5
8.6	0	0	+	1.2	2.0	3.1	3.5	4.4	4.5	2.2	1.3
9.2	0	0	+	0.7	1.1	2.2	2.8	3.4	3.5	1.8	0.3

mined by growing the fungus on potato-dextrose agar. Enough agar for the entire experiment was prepared in one batch and adjusted to the desired pH as it cooled after sterilization. The agar plates were inoculated by taking small squares from a plate culture previously prepared. The rate of growth was determined by measuring the diameter of colonies produced during a period of 5 days. The values given in table 3 represent the average diameter of growth recorded from three cultures.

The optimum temperature for growth of this fungus on potato-dextrose agar was found to be 24–27° C. (Fig. 4), while the optimum reaction of the agar was on the acid side of the neutral point. The influence of the reaction

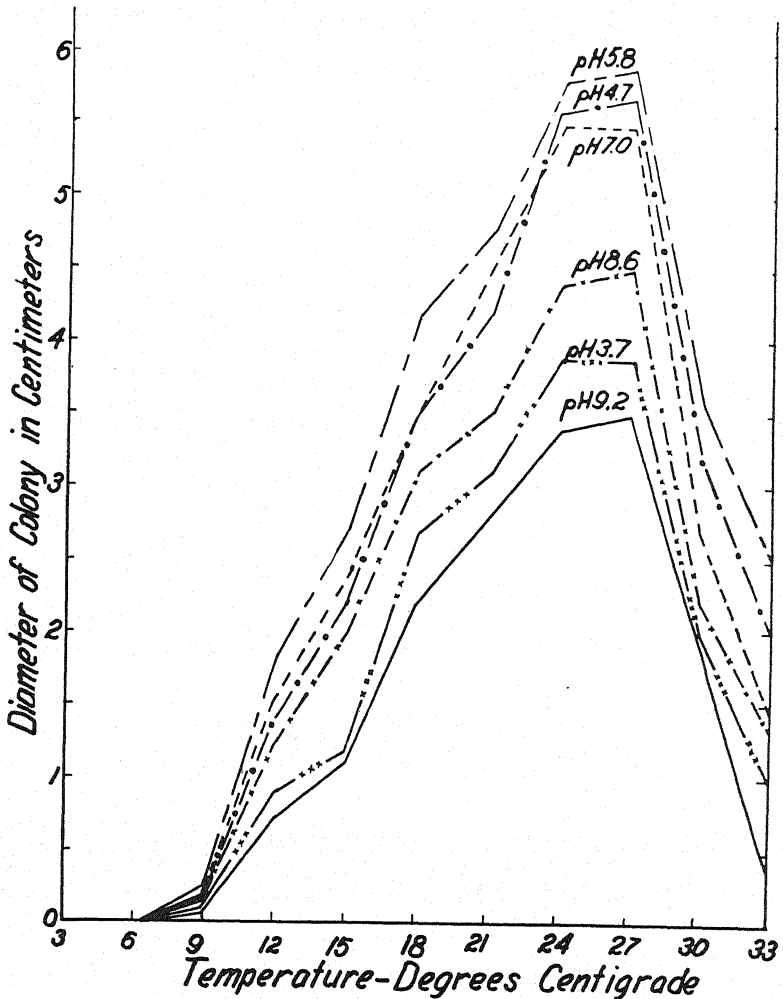


FIG. 4. Growth of *Fusarium conglutinans* var. *betae* on potato-dextrose agar.

of the substratum is of interest, since most soils where the disease has been found are neutral to alkaline in reaction.

VIRULENCE OF THE PATHOGENE

The amount of disease produced in seedlings by *Fusarium conglutinans* var. *betae* was compared with *Corticium vagum* B. and C. (*Rhizoctonia solani* Kuhn) and *Pythium debaryanum* Hesse. The soil, a sandy loam, was sterilized by steaming under 15 pounds pressure for 4 hours. The organisms were grown in large quantities on sterile wheat kernels and transferred to the soil about a week before planting the seed. The seed balls were pasteurized at 60° F. for 10 minutes on three consecutive days to eliminate seed-borne organisms. Fifty seed balls were planted to a 5-inch pot. The seedling counts given in table 4 represent a 14-day period after the plants began to emerge.

Isolations were made from one or more diseased seedlings from day to day and in every case only the organism with which the soil had been inoculated was obtained.

The conditions of this test were very favorable for the production of seedling disease, as demonstrated by the failure of a single seedling to get

TABLE 4.—*Disease produced in seedlings by certain soil-inhabiting pathogenes of sugar beets*

	Pot	Plants emerging		Amount of disease	
		Total	Plants per seed ball	No. plants	Per cent
Check	A	62	1.24	0	0
	B	71	1.42	0	0
	C	66	1.32	0	0
	D	70	1.40	0	0
		67.25	1.345		
Fusarium conglutinans var. *betae*	A	60	1.20	49	81.7
	B	72	1.44	56	77.8
	C	59	1.18	46	78.0
	D	73	1.46	61	83.6
		66	1.320		80.3
Pythium debaryanum	A	5	.10	5	100
	B	18	.36	18	100
	C	11	.22	11	100
	D	10	.20	10	100.0
		11	.22		
Rhizoctonia solani	A	0	0	0	0
	B	0	0	0	0
	C	0	0	0	0
	D	0	0	0	0

above the surface in the soil inoculated with *Rhizoctonia solani*. The organism was readily isolated from the seed balls. In the case of *Pythium debaryanum* a few seedlings emerged but they were all diseased before the end of the 14-day period. It is interesting to note that the total of plants emerging in the soil inoculated with *Fusarium conglutinans* var. *betae* was very similar to that in the sterilized soil; however, within 14 days 80 per cent of the plants had become diseased. From these data it is evident that *F. conglutinans* var. *betae* can be classed as a virulent pathogene of sugar beets but a slower-acting organism than *R. solani* or *Pythium debaryanum*.

Plants affected. The organism causing beet yellows is known to produce disease only in beets. It has occurred in fields used for variety testing but not in sufficient amounts to enable one to ascertain varietal differences in susceptibility. Various crop plants, such as lima beans, string beans, cantaloupes, cucumbers, alfalfa, cabbage, and asters, grown in the region where the disease is known to occur, were planted in infested soil or inoculated by inserting the organism into the stems of plants; however, none of the plants so treated became diseased.

SUMMARY

A new disease of sugar beets has been found to occur in the beet fields of Colorado, which is characterized by the production of a yellowing of the leaves and a gray dry rot of the vascular system of the roots.

It is important due to the marked reduction in the percentage of sugar and weight in the diseased plants as well as the loss of individuals during the summer.

The pathogene appears to be new to literature and is here provisionally named *Fusarium conglutinans* var. *betae*.

The optimum temperature for growth of the pathogene on potato-dextrose agar was found to be 24–27° C. The greatest growth occurred at pH 5.8 of the hydrogen-ion concentrations used.

The pathogene can be classified as a virulent one on seedlings, but its lethal effect is delayed as compared with that of *Rhizoctonia solani* or *Pythium debaryanum*.

OFFICE OF SUGAR PLANTS,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

ALFALFA DWARF, A HITHERTO UNREPORTED DISEASE[1]

J. L. WEIMER[2]

INTRODUCTION

For the last few years alfalfa growers in Calfornia, like those in many other States, have experienced difficulty in maintaining their stands of alfalfa for a satisfactory period of years. This fact was brought to the writer's attention in 1927 by B. A. Madson of the University of California. A study of diseased alfalfa roots sent by Professor Madson to F. R. Jones and the writer led to the conclusion that the trouble in some parts of California was due to the bacterial wilt caused by *Phytomonas insidiosa* (McC.) Bergey *et al.* However, diseased plants from other parts of the State, although exhibiting somewhat similar symptoms, did not appear to have the wilt bacteria present. Aside from difficulties primarily due to cultural and soil factors, which it is not the purpose of this paper to discuss, further study by the writer has shown that, the shortening of the life of the alfalfa stands in California can be attributed to two causes. Throughout the San Joaquin Valley, at least from Bakersfield to Modesto, bacterial wilt is largely responsible for the premature depletion of the alfalfa stands. This disease has been reported also from the Sacramento Valley. On the other hand, wilt is rarely found south of the Tehachapi Mountains, but another disease possessing some of the characteristics of wilt is very prevalent and destructive there. It is the purpose of this paper to give a brief preliminary report on this latter disease, which, so far as the writer is aware, has not been described hitherto.

THE DISEASE

Name. Plants affected with the disease under discussion gradually become smaller until they finally succumb without presenting any other striking top symptom; hence the name "alfalfa dwarf" is suggested. To the writer's knowledge the only name that has been applied to this trouble by growers is "little leaf." However, since the stems also become short and slender the name "dwarf" seems more descriptive of the symptoms.

History and Geographic Distribution. When or where the disease originated is not known. Many farmers state that until about 10 or 12

[1] Cooperative investigations between the California Agricultural Experiment Station and the Office of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Senior Pathologist, Office of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

years ago they had no difficulty in maintaining satisfactory stands of alfalfa for 8 to 10 years or longer, whereas at the present time a stand is seldom worth maintaining for more than 3 years in sections where the disease is most destructive.

In a discussion of the status of alfalfa in the Riverside area, Nelson and associates[3] state that the yields of alfalfa on sandy land frequently begin to decline somewhat after the fourth year and that the crop often becomes unprofitable after the seventh or eighth year. Since no mention is made of a decline of yields on the heavier soils, it seems quite probable that no general trouble such as exists at the present time was being experienced then (1915). A survey of the alfalfa sections of southern California, made by B. A. Madson during the summer of 1927, showed that at that time the stands remained productive for only two to four years.[4]

No evidence of the existence of this disease outside of southern California is at hand. However, no extensive survey has been made to determine the limits of its distribution.

Economic Importance. Present information indicates that the dwarf disease is largely responsible for the short-lived stands of alfalfa in southern California. In some sections the disease limits the life of the stand to two years, while in others a satisfactory crop is harvested for a period of four years or longer. Certain regions, such as the Mojave Desert section of San Bernardino County, the Hemet Valley, and a few other places, suffer little or not at all from the disease. In some of these sections the disease seems very limited in its distribution, while in others it is widespread but apparently held in check largely by cultural conditions.

The disease not only shortens the life of the stand but cuts down the yield materially, especially during the latter part of the last year of the stand. Fields have been seen in which nearly every plant was affected, the stand and the yield being greatly reduced. No doubt this disease causes a loss of thousands of dollars annually.

The disease may be distributed more or less uniformly in the field, or it may be limited to certain patches. The latter is more commonly the case, at least in the early stages. Most frequently it appears first along irrigation ditches or where the soil moisture is highest and spreads from these areas until most or all of the field is involved.

Symptoms. The earliest stages of alfalfa dwarf can not be detected by the aboveground symptoms, since the disease is already well advanced

[3] NELSON, J. W., R. L. PENDLETON, J. E. DUNN, A. T. STRAHORN, and E. B. WATSON. SOIL SURVEY OF THE RIVERSIDE AREA, CALIFORNIA. U. S. Dept. Agr., Bur. Soils Field Oper. 1915 (Rpt. 17.): 2367–2450. 1919.

[4] MERRILL, E. D. REPORT OF THE DIRECTOR OF THE AGRICULTURAL EXPERIMENT STATION. Calif. Agr. Exp. Sta. Ann. Rpt. 1926/27. 1927.

in the root before it becomes evident in the top. The first signs of the disease in the tops, are a shortening of the stems and a slight reduction in the size of the leaves. Blossoming is often retarded or inhibited. After each cutting the stems of affected plants become shorter and slenderer and the leaves smaller. Likewise, fewer new buds are developed each time, resulting in a gradual diminution of the number of stems. Usually, no chlorosis or other color change occurs in the leaves or stems until the last few stems die. In the final stages of the disease only one or at most a very few stems are produced, and these reach an ultimate height of but a few inches. These stems remain upright and for the most part turgid until death ensues. Not infrequently the leaves of diseased plants have appeared to be of darker green than those of neighboring healthy plants. The leaves of affected plants are not mottled, crinkled, or deformed, although commonly they are somewhat rounded at their apices, resembling more closely the basal than the terminal leaves of healthy plants. Although not of very common occurrence, wilting of the tips of the stems may occur in the later stages of the disease even in quite wet soil. The stems of diseased plants usually are reduced more or less uniformly in size. (Fig. 1.)

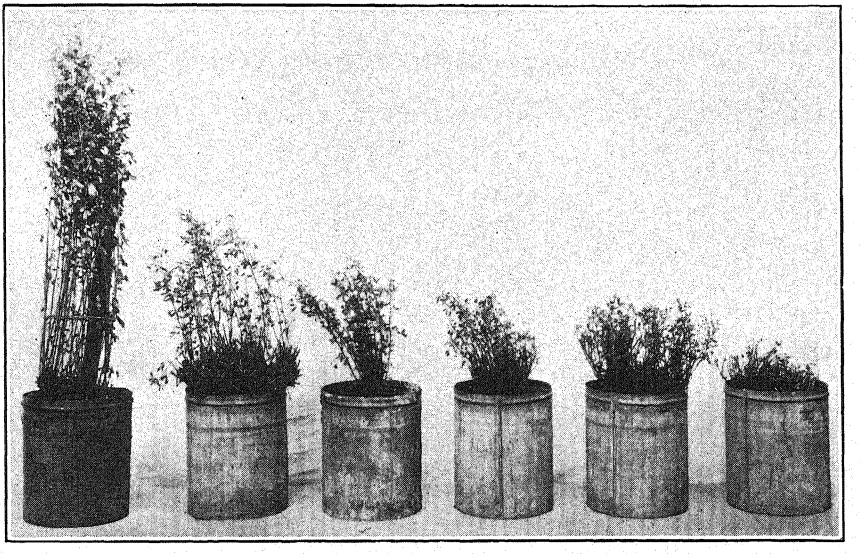


FIG. 1. Alfalfa plants of the Chilean variety affected with the dwarf disease. The healthy plant at the left is of the same age and from the same part of the field as the other plants, which are diseased. Note especially the slender stems of the diseased plants as compared with those of the healthy, and the gradual diminution in height. The plants were removed from the field just before being photographed.

The first evidence of this disease in the root thus far observed is a small yellow streak in the wood, apparent only when the bark is removed. This streak, which may be from a few millimeters to several centimeters long by a few millimeters wide, may occur anywhere in the root, especially in the upper foot of the taproot. As the disease develops, the yellowing spreads until it eventually involves the entire circumference of the root. As far as observed, yellowing is never present in the bark unless secondary infection has begun. When the root is cut across, the yellow color is found in the outermost part of the woody cylinder just beneath the bark. This discolored tissue forms a definite ring or band, which is narrow at first but becomes wider as the disease develops, until at the time the plant dies the root is frequently discolored throughout its entire diameter. How much of this discoloring is due to secondary causes is not known. Plants may die while the band of yellow tissue is comparatively narrow, but in this case it extends to the cambium. This condition is commonly seen during the hot summer months when the plants are dying most rapidly. During the winter and early spring months the yellowing usually does not extend to the cambium but is buried beneath a thin layer of new xylem, indicating that the disease it not active during the winter months. The xylem of the secondary and tertiary roots also becomes yellow, and death ensues. Sometimes small roots are formed near the crown, which support a more vigorous top growth than seems possible, considering the condition of the main taproot. The yellowing extends into the main divisions of the crown and into the base of the green stems, but thus far it has not been found to go very far up into the latter.

The yellowing in the roots of plants affected with dwarf, being much lighter in color, is distinctly different from that produced by the Fusarium wilt described by Weimer.[5]

Plants with slender stems and small leaves resembling those of plants affected with the dwarf disease have been seen in fields suffering from drought or from some soil deficiency, but such plants do not show the yellowing in the roots. The roots as well as the tops of such plants are usually small, whereas the roots of plants affected with dwarf frequently are the largest and most vigorous in appearance of any others in the particular area in which they are found.

COMPARISON OF ALFALFA DWARF AND BACTERIAL WILT

Since the symptoms produced by the dwarf disease and by bacterial wilt are so similar that it is often difficult for one not familiar with both diseases to tell them apart, it seems desirable to compare rather definitely

[5] WEIMER, J. L. A WILT DISEASE OF ALFALFA CAUSED BY *Fusarium oxysporum* VAR. *medicaginis*, N. VAR. Jour. Agr. Res. 37: 419–433. 1928.

the symptoms of each. The following points should be helpful in distinguishing between these two diseases.

(1) Both wilt and dwarf cause a decided stunting of the tops of the plants as the diseases progress, although neither produces evident top symptoms in the earliest stages.

(2) In both diseases the stems become fewer, shorter, and more spindling after each cutting, until only a very few stems are produced, and these eventually die.

(3) Bacterial wilt causes stunted and abnormally shaped leaves that are usually paler in color than those of healthy plants. In the dwarf disease the leaves become quite small but remain practically normal in shape and color until the death of the plant.

(4) Both diseases may produce wilting under certain conditions, but this is not a constant or very conspicuous symptom of either disease.

(5) The root symptoms produced by the two diseases are very similar. The earliest stage that can be detected in roots affected with either disease is a slight yellowing of the wood just beneath the bark. This yellowing, which results largely from the formation of gum in the vessels, spreads until the entire active part of the xylem is more or less completely involved. The reddish-brown lesions in the bark and wood of roots affected with wilt, described and illustrated by Jones and McCulloch,[6] have never been seen in roots affected with dwarf.

(6) In case of doubt the presence of the bacteria in the vessels of the roots of plants affected with wilt can easily be demonstrated in freehand sections by Gram's stain. No bacteria will be seen in similarly treated sections of roots having the dwarf disease.

SUMMARY

A brief preliminary description of a hitherto underscribed disease of alfalfa occurring in southern California is given. The symptoms of this disease are quite similar to those of bacterial wilt; hence the similarities and differences between the two are pointed out.

[6] JONES, F. R., and L. MCCULLOCH. A BACTERIAL WILT AND ROOT ROT OF ALFALFA CAUSED BY *Aplanobacter insidiosum* L. McC. Jour. Agr. Res. 33: 493–521. 1926.

DIAPORTHE BLIGHT OF LARKSPUR

FREDERICK A. WOLF

Larkspur, *Delphinium ajacis* L., within the United States, is subject to several diseases two of which are more or less well known, sclerotial blight caused by *Sclerotium delphinii* Welch (5, 3), and bacterial leaf spot caused by *Bacterium delphinii* (E. F. S.) Bryan (1). The former appears as a decay of the cortical portion of the stems at or near the ground level, eventuating in a girdling and death of the plants. The presence of sclerotia in a mycelial weft near the base of the stem constitutes a characteristic sign of this disease. The latter may be recognized by the presence of dark brown to blackish spots on the leaves and streaks on the stems.

Another disease, apparently undescribed, and one that may be appropriately designated "Diaporthe blight," was first observed in the vicinity of Durham, N. C., during the spring of 1929. Attention is first directed to this disease because of the fact that the lower leaves of plants which have reached the flowering stage become brown and dry and remain attached. Closer examination shows that brown lesions occur near the base of the stems. By the time that these lesions have developed to the extent of completely girdling the stems, the entire foliage will have gradually withered and become dry. Eventually the lesions extend upward several inches above the surface of the soil and downward into the root system. Scattered dark pycnidia are present within the stems (Fig. A) and within the petioles and leaf blades of the lowermost leaves before the plants have succumbed, and new pycnidia continue to be formed for a considerable period thereafter. The crown and uppermost roots are enveloped in a cottony weft of mycelium during rainy periods.

Toward the close of the growing season scattered pycnidia appear upon the capsules. Such capsules are in some cases blighted and fail to mature and in others apparently normal seed are borne in affected capsules. The seed from diseased capsules, in all likelihood, serve as a means of initiating this blight in new localities.

Microscopic examination shows that the spherical to ellipsoidal pycnidia which measure 100 to 150 μ are wholly immersed within the tissues. They are of the Phomopsis type, possess a wall of dark cells, and contain a single locule from which the conidia are exuded by a pore. They bear two kinds of hyaline conidia, alpha conidia (Fig. B, 3), which are capable of germination, and beta conidia or stylospores (Fig. B, 2), which have never been noted to be capable of germination. The former are developed less abundantly both on larkspur stems and in culture. They are oval and

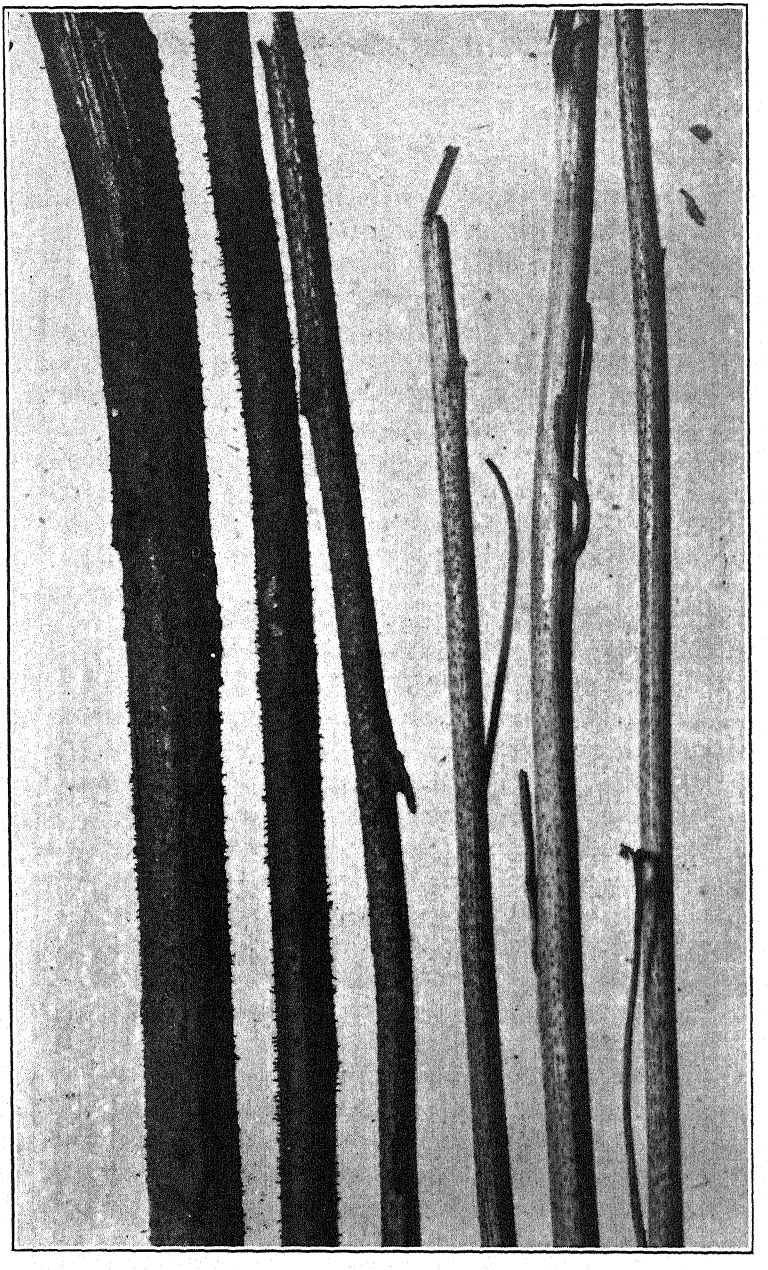


FIG. A. At the left, three stems of larkspur bearing the conidial stage of *Diaporthe arctii*, and, at the right, three bearing the ascigerous stage.

measure 8–10 by 3.54 μ, while the latter are thread-like and hooked or curved and measure 20–30 by 0.75 μ.

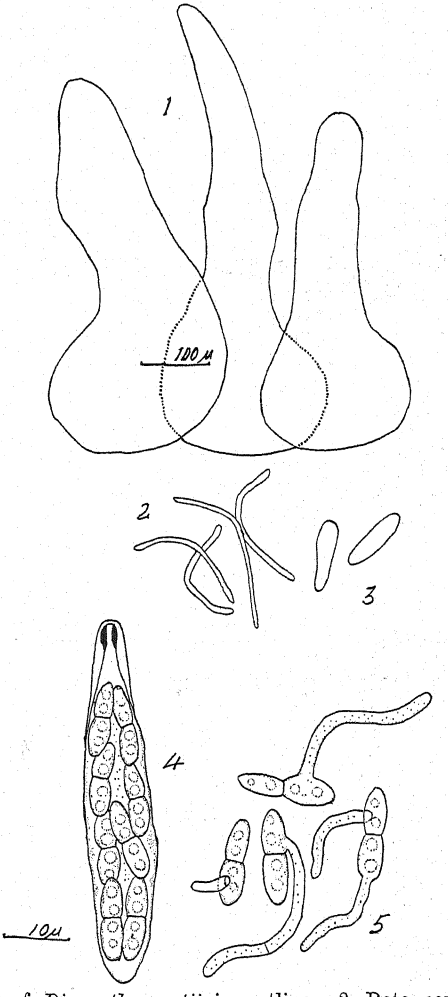


FIG. B. 1. Perithecia of *Diaporthe arctii* in outline. 2. Beta conidia or stylospores. 3. Alpha conidia. 4. Typical ascus showing arrangement of ascospores and apical pore. 5. Germination of ascospores on agar after 15 hours.

A bundle of blighted stems was collected during May, 1929, and allowed to remain out of doors in contact with the soil until the following March. In the interim the surface of the stems had been blackened and a perithecial stage of the Diaporthe type had developed (Fig. A). There is no well-defined ventral zone. The perithecia occur singly, are sunken within

the substratum, and extend to the surface by means of thick, elongated beaks 300 to 400 μ long (Fig. B, 1). The diameter of their basal portion is 200 to 225 μ and of their beaks 75 to 100 μ. The asci are 45–50 by 9–10 μ, with a refringent apical pore. The ascospores are hyaline, one-septate, guttulate, are arranged biseriately, and measure 10 to 12 by 3 to 3.5 μ (Fig. B, 4).

Isolations were made by taking advantage of the fact that the ascospores are forcibly expelled. Stems bearing perithecia were placed on moist blotting paper in the tops of inverted agar plates. Within a few hours great numbers of the ascospores were found to have adhered to the surface of the agar above. When the ascospores were transfered to tubes of potato agar or to sterilized larkspur stems, the colonies which developed consisted of a loose white mycelium. After a period of about two weeks, Phomopsis pycnidia had formed sparingly in these colonies.

Additional evidence of the genetic connection of the perithecial and conidial stage and, at the same time, of pathogenicity was obtained by series of inoculations. In one series the inoculum consisted of drops of water containing macerated perithecia; in another, a suspension of conidia from pure cultures isolated from ascospores. Small brown lesions were apparent on all inoculated plants 7 to 8 days after inoculation, and Phomopsis pycnidia had formed within the succeeding 10-day period. The larkspur-blight organism is, therefore, another Diaporthe of the group whose conidial stage is parasitic and whose perithecial stage develops on dead or decaying parts, typified by such well-known pathogenes as *D. phaseolarum* (C. & E.) Ell., *D. batatatis* Harter and Field, and *D. citri* (Fawcett) Wolf.

The question of the identity of the larkspur-blight fungus revolves around whether one should regard it as specifically identical with previously described forms on the basis of morphologic resemblance to such species, or whether one should emphasize its distinctness on the basis of suscept relationships. Many species of Diaporthe are known to be limited to closely related suscept species, a fact which has served as a means of identification of species which are morphologically alike or are very similar to previously described forms on other kinds of plants. Recently, Wehmeyer (4) directed attention to the influence of substrate on species of Diaporthe. He considered the effect of substratum on the character of the stromata and on the size and shape of the perithecial and conidial stages. He concludes that in some species stromatic characters are quite variable; in others, more or less constant; and that morphologic differences in perithecial stages and conidial stages are correlated with the genus or species of plant upon which the fungus is grown. He suggests furthermore that "species groups" may, in subsequent studies, be separated.

Apparently no species of Diaporthe, as has been stated, has been previously described on Delphinium. When, however, the larkspur pathogene is compared with the numerous forms described by Nitschke (2) it is found to correspond morphologically with *D. arctii* (Lasch) Nit.[1] This is a group species known to occur on stems of Lappa, Circium, Carduus, Tannecetum, and Centaurea. Nitschke states that a fungus on *Carduus acanthoides* which is labelled *Sphaeria orthoceras = Sphaeria achilleae* should be *D. arctii.* Auserwald described a *D. achilleae* on *Achillea millefolium* which he later identified as *D. orthoceras* Fr. The organism which Nitschke used as the basis of his *D. orthoceras* (Fr.) Nit. occurs on Achillea. However, Fries's *Sphaeria orthoceras* (Elenchus Fungorum II, p. 97), specimens of which were not available to Nitschke, occurs on Lapsana. If one emphasizes the importance of substrate these could all be distinct. If, on the other hand, one regards the slight differences to be the result of substrate, as indicated by Wehmeyer's studies (4) on Diaporthe, and as is most reasonable at the present state of our knowledge of this genus and other sphaeriaceous fungi, then they should all be regarded as one and the same. The writer therefore regards the larkspur pathogene as *D. arctii.*

SUMMARY

A brief account is given of a blight of larkspur caused by *Diaporthe arctii.* The pathogene possesses a Phomopsis stage, which appears on living parts, and an ascigerous stage, which develops on decaying stems. The genetic connection of these stages is established by cultures and by inoculation. Infections resulted from inoculation with ascospores and with conidia from culture from ascospores.

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[1] Thanks for this identification are due to Dr. C. L. Shear, U. S. Department of Agriculture, Washington, D. C., and to Dr. L. E. Wehmeyer, Ann Arbor, Mich., to whom specimens were submitted.

EFFECT OF SHADING ON THE RATE OF DEVELOPMENT OF TOMATO YELLOWS[1]

MICHAEL SHAPOVALOV AND J. W. LESLEY

It has been frequently assumed that the percentage of plants affected by curly top in sugar beets and by yellows in tomatoes depends simply on the degree of infestation with the beet leaf hopper (*Eutettix tenellus* Baker) which spreads these diseases. Variations in the geographical and seasonal distribution of tomato yellows have been explained as merely determined by variations in the prevalence of the hopper in different sections and seasons. The common observation that tomato yellows is less severe in orchards or in other shady places than in adjoining open fields has been understood to mean that the insect vector loves sunshine and abhors the shade. Since the beet leaf hopper is the only known carrier of the curly-top virus in the United States, unquestionably a correlation between the degree of infestation with the leaf hopper and the percentage of infection is to be expected. Nevertheless, some of the recent infection trials[2] have indicated very clearly that the development of the disease is also determined by the conditions to which the plant is exposed after inoculation, particularly by the intensity of light. When potted plants, all inoculated by the same method and otherwise treated in an identical manner, were placed under different light intensities, a greater proportion remained healthy when heavily shaded than when lightly shaded or not shaded at all. Our field trials at Shafter, California, in 1929, with plants exposed only to natural infection, present additional strong evidence of this. The progress of yellows in infected plants was delayed by shading, but the symptoms developed more rapidly after shading was discontinued.

The Stone variety of tomato and two closely related dwarf hybrid progenies were used in these trials. The plat consisted of two rows of unshaded Stone, two rows of unshaded dwarfs, and two rows of shaded dwarfs. It was subject to natural infection only. The shade was afforded by muslin tents extending over entire rows, fully open at the ends and with considerable openings on the sides, as shown in figure 1. Thus the intensity of light was reduced for the sheltered rows, although insect invasion of the tents was not entirely prevented.

[1] Joint contribution from the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C., and the University of California. Paper No. 227, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Shapovalov, Michael, and F. Sidney Beecher. Experiments on the control of tomato yellows. U. S. Dept. Agr. Tech. Bul. 189. 1930.

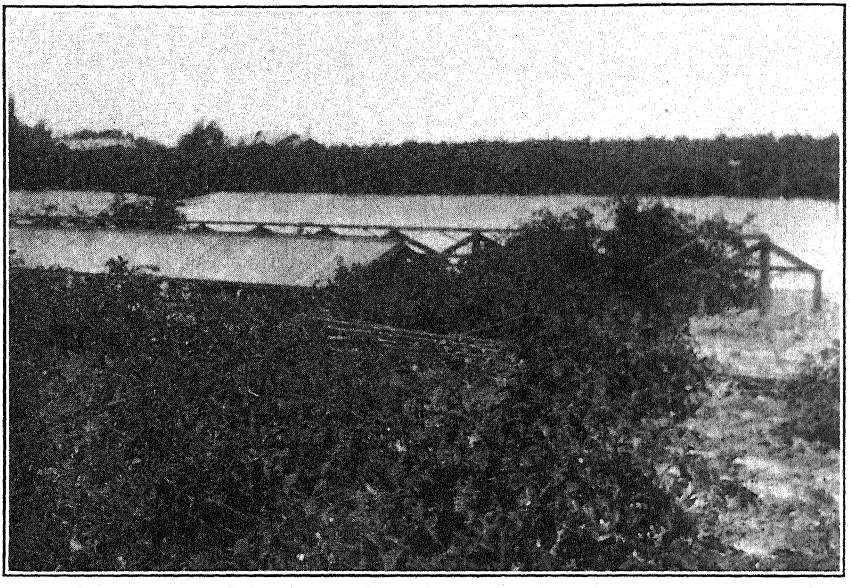


FIG. 1. The type of tents used at Shafter, Calif., in shading experiments with tomatoes.

As shown in table 1, unshaded rows of both varieties, the majority of the season's total number of cases of yellows had developed by June 11 and only a very small additional proportion by July 8 or later. In shaded rows no indication of yellows whatever was noticed until June 11 and subsequently for some days the development of the disease was slow. On July 5 the tents were removed. Three days later, on July 8, the number of affected plants in the two shaded rows had increased by 90 per cent and on July 26 by more than 100 per cent over that noted 11 days prior to the removal of the tents. In the unshaded rows, the rate of increase of yellows was much greater in the period June 11–24 (13 days) than in the period June 24–July 8 (14 days), whereas in the shaded rows the reverse occurred. These differences are shown in figure 2, where each line represents averages of the two rows of each kind.

These results indicate very clearly that many of the apparently healthy plants had become infected by invading leaf hoppers while these plants were still shaded by the tents. The environmental conditions under the tents were beneficial to the host plants and unfavorable to the virus. It is quite possible that the entire amount of infection in the shaded rows occurred on the same days as in the unshaded rows and that the delayed appearance of the symptoms of yellows under the tents signified merely a slower development of the disease. If the sudden increase of yellows after June 24 in the shaded rows was caused by new infection, there should have

TABLE 1.—*Progress of tomato yellows in shaded and unshaded rows at Shafter, Calif., in 1929*

Variety and treament	Total number of plants	Number and per cent of plants affected by yellows from the beginning of season up to dates shown below.													
		May 21		*May 31*		*June 11*		*June 17*		*June 24*		*July 8*		*July 26*		*August 2*	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Stone Unshaded	51	1	2.0	30	58.8	38	74.5	38	74.5	41	80.4	41	80.4	43	84.3	43	84.3
Hybrid 006.18 Unshaded	51	1	2.0	15	29.4	27	52.9	31	60.8	32	62.7	32	62.7	35	68.6	35	68.6
" Shaded	50	0	0.0	0	0.0	4	8.0	8	16.0	8	16.0	18	36.0	27	54.0	30	60.0
Stone Unshaded	50	5	10.0	12	24.0	21	42.0	25	50.0	28	56.0	31	62.0	34	68.0	35	70.0
Hybrid 006.24 Unshaded	51	2	4.0	9	17.7	17	33.3	22	43.1	24	47.1	25	49.0	26	51.0	26	51.0
" Shaded	49	0	0.0	0	0.0	5	10.1	5	10.1	11	22.4	18	36.7	20	40.8	23	47.0

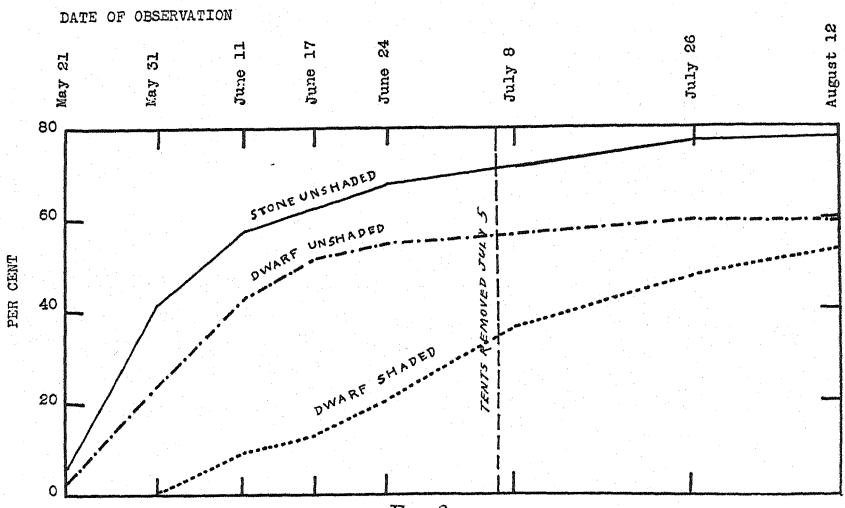


FIG. 2.

been a corresponding simultaneous increase in the unprotected rows, but such was not the case. Over 90 per cent of the season's total of yellows developed in the unshaded rows by the end of June. This is entirely in line with what might have been expected from previous experience under the conditions at Shafter. Certain counts taken there during the last week in June showed 98 per cent of yellows in 1926 and 95 per cent in 1927 and

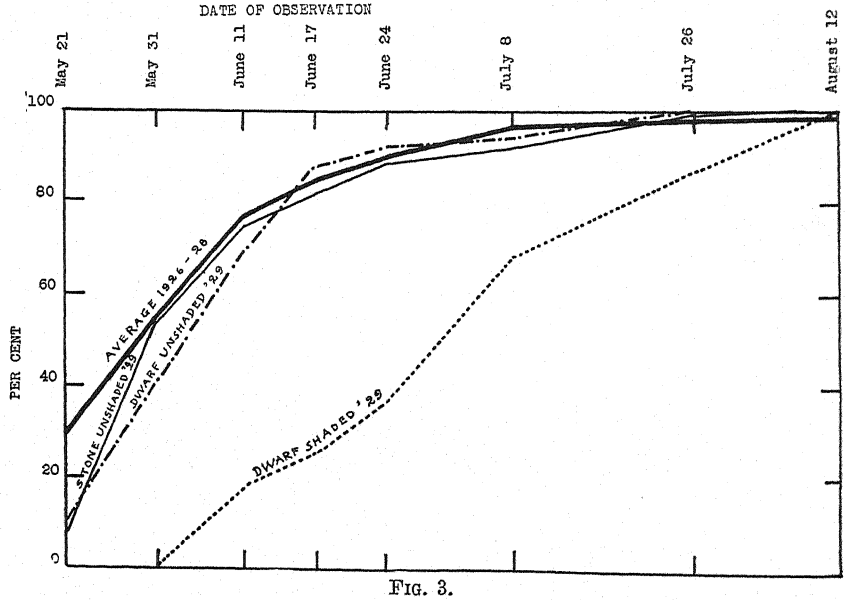


FIG. 3.

1928 in unshaded rows. The progress of the disease for these years is illustrated in figure 3. Furthermore, the period of 3 days between the removal of the tents and the next inspection on July 8 is far too short for the new infection to develop distinct symptoms of yellows in plants which had been growing for three months in the field. In previous inoculation field trials with viruliferous leaf hoppers (*E. tenellus*) only a very small percentage of very young plants (2 to 3 inches in height) showed early symptoms of yellows on the fourth day after the removal of the insects. Larger plants require a much longer incubation period. At Riverside, plants inoculated 3 weeks after transplanting required at least 16 days before the symptoms could be detected. Therefore, we may safely infer that most of the shaded plants became infected before the middle of June, as was apparently the case with the unshaded rows, and that the three days' exposure to the climatic conditions at Shafter in July following the removal of the tents, in addition to over two weeks of incubation under shelters, during which diffusion of the virus probably occurred, was sufficient to bring out the characteristic symptoms of yellows in those plants in which the virus was present.

It should be concluded from the foregoing data and discussion that proper shading of tomato plants in the regions characterized by the prevalence of the beet leaf hoppers only partially protects the plants from the invasion of the infecting hoppers and that it increases the tolerance of the plants to the virus, enabling them to produce a crop if the shading is continued after the infection takes place and may help some plants entirely to overcome it.

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NATURAL FIRE-BLIGHT INFECTIONS ON SPIRAEA VANHOUTTEI

A. B. Groves

The natural occurrence of fire blight on *Spiraea Vanhouttei* was first suspected by Rosen and Groves (1) after artificial infections were readily produced with pure-culture suspensions of *Bacillus amylovorus* (Burr.) Trev. The possibility of observing any natural infections then was rendered difficult by the prevalence of frost injury to the specimens available.

The most favorable conditions for the occurrence of natural fire-blight infections on *Spiraea Vanhouttei* existed at the Winchester Research Laboratory of the Virginia Agricultural Experiment Station, where there were two specimens located directly beneath a large pear tree. (Fig. 1). These were examined frequently to locate any infections that might occur under such favorable conditions. The first of what appeared to be fire blight on the Spiraea was observed on May 12, three days after the appearance of fire blight on the pear tree beneath which they were located. By this time the disease was general throughout the tree, particularly on the water sprouts which were abundant on the larger limbs. Several of the diseased twigs (Fig. 2) were removed and isolations attempted from them, together with one of the blighted pear twigs. Bacteria were obtained in abundance from all the material, the colonies being typical of *Bacillus amylovorus*. Transfers were made from these and suspensions in sterile distilled water were prepared from the transfers when 48 hours old. These suspensions were used in making inoculations into both young pear and Spiraea shoots which had been removed and placed with the cut ends in water in the laboratory. This precaution was taken to prevent possible external natural infection. The bacteria obtained from the blighted Spiraea produced typical blight on the pear and Spiraea shoots, as did the bacteria obtained from the pear. The first evidence of blighting from these infections was visible 40 hours after inoculation. The organism was readily recovered. The disease produced on the artifically infected Spiraea was very similar to that described by Rosen and Groves,[1] the shoot proper becoming flaccid and discolored, without apparent invasion of the leaves attached. Small droplets of ooze did appear, however. The disease advanced somewhat more vigorously on the inoculated shoots than on the naturally infected ones.

[1] Rosen, H. R., and A. B. Groves. Studies on fire blight: host range. Jour. Agr. Res. 37: 493–505. 1928.

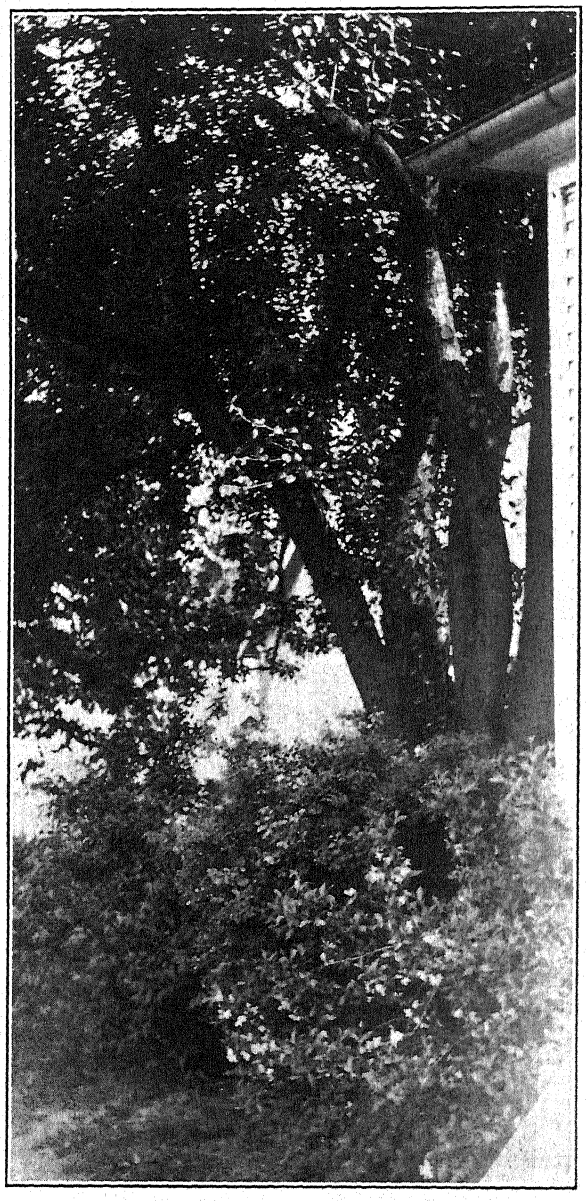


FIG. 1. Photograph showing the location of a *Spiraea Vanhouttei* plant directly beneath a pear tree. The numerous water sprouts may be observed, a number of which are blighted.

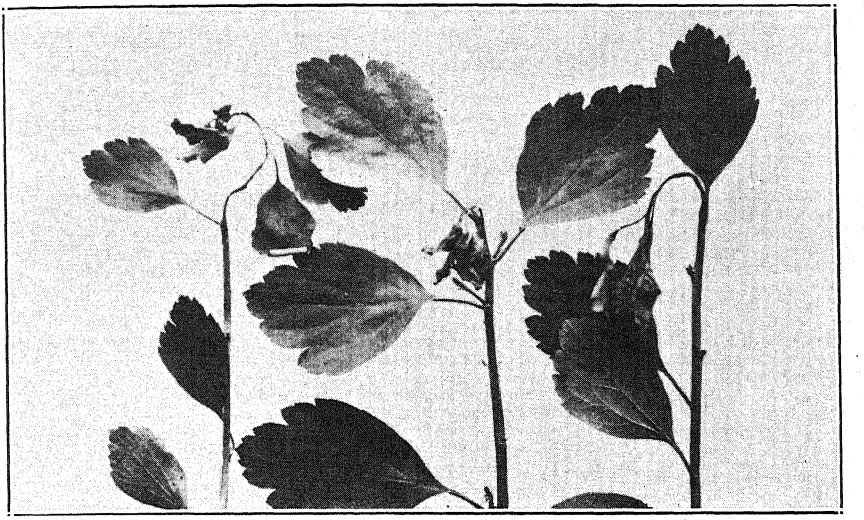


FIG. 2. Blighted shoots of *Spiraea Vanhouttei*, naturally infected.

A number of Spiraea plants are located in the planting about the building, but blight was found only on those directly beneath the blighting pear tree. The foregoing indicates that natural infections of fire blight do occur on *Spiraea Vanhouttei* under conditions favorable for such infections, as herein noted.

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CERCOSPORA BATATICOLA, N. SP., PARASITE OF THE SWEET POTATO IN AMERICA

R. CIFERRI AND S. C. BRUNER

Cercospora batatae Zimmerman,[1] parasitic on the sweet potato, *Ipomoea batatas* L., was originally described from material of African origin and later reported from other countries of the oriental region, as has been noted in the work of Harter and Weimer.[2] A Cercospora is known also to occur on the sweet potato in America, and these authors call attention to the fact that the species found in Florida is possibly distinct from that of the Old World.

Through the courtesy of Dr. G. F. Weber, plant pathologist of the Agricultural Experiment Station of the University of Florida, we have obtained a specimen of the Cercospora of Florida which has been studied in conjunction with others from Cuba and Santo Domingo collected by the writers, and these, in turn, compared with a specimen from the Philippines obtained through exchange.

The results of a biometrical study of the conidia, measured by means of a filar micrometer and Bausch and Lomb 7.9 and 4 mm. objectives, are given in the following tables.

TABLE 1.—*Measurements of length of conidia of the American and Philippine species of Cercospora*

Class, microns	Florida	Cuba	Santo Domingo	Recapitulation 3 species	Philippines
41–50	1	2	0	3	1
51–60	2	4	2	8	2
61–70	1	6	2	9	9
71–80	3	9	3	15	14
81–90	7	4	12	23	6
91–100	10	4	7	21	2
101–110	6	2	4	12	1
111–120	2	2	3	7	0
121–130	1	1	2	4	0
131–140	0	1	0	1	0
141–150	1	0	0	1	0
151–160	1	0	0	1	0
Total	35	35	35	105	35

[1] Zimmerman, A. *Untersuchungen über tropische pflanzenkrankheiten,* Erste Mitteil. Ber. Land. u. Forstw. Deutsche Ostafrika 2: 11–36. 1904.

[2] Harter, L. L., and J. L. Weimer. *A monographic study of sweet-potato diseases and their control.* U. S. Dept. Agr. Tech. Bul. 99. 1929.

TABLE 2.—*Measurements of width of conidia of the American and Philippine species of Cercospora*

Class, microns	Florida	Cuba	Santo Domingo	Recapitulation	Philippines	Total
2	0	1	0	1	1	3
3	3	5	2	10	11	31
4	13	20	7	40	19	99
5	17	8	22	47	3	97
6	2	1	4	7	1	15

TABLE 3.—*Number of septae characterizing the conidia of the American and Philippine species of Cercospora*

Class, septae	Florida	Cuba	Santo Domingo	Recapitulation	Philippines	Total
2	0	0	0	0	1	1
3	0	0	0	0	4	4
4	0	1	0	1	8	10
5	1	1	0	2	10	14
6	2	2	1	5	6	16
7	1	4	1	6	3	15
8	3	8	2	13	2	28
9	4	9	2	15	0	30
10	7	6	10	23	1	67
11	5	2	10	17	0	34
12	7	1	5	13	0	26
13	1	1	2	4	0	8
14	2	0	1	3	0	6
15	1	0	1	2	0	4
16	1	0	0	1	0	2

The specimens recorded above were collected as follows:

1. Florida. Labeled: *Cercospora* sp. not *batatae*. On *Ipomoea batatas*. Homestead, Florida, 1, 23, 23. Coll. G. F. Weber.

2. Cuba. Labeled: *Cercospora* sp. on *Ipomoea batatas*, Wajay, Habana, 9, 26, 29. Coll. S. C. Bruner.

3. Santo Domingo. Labeled: *Cercospora* sp. On *Ipomoea batatas*. Colonia Jamao, Moca, 5, 14, 28. Coll. R. Ciferri.

4. Philippines. Labeled: *Cercospora batatae* Zimm. On *Ipomoea batatas* L., Los Baños, 16, 6, 19. Coll. ?

Although the Cercospora found on sweet potato in America shows considerable biometrical variation, yet, judging from the average measurements of the three sets of specimens studied, it appears to be distinct from the

Asiatic species in having longer and broader conidia and in being provided with a larger number of septae.

No difference was seen in the form of the conidia, which in all of the specimens is elliptic-elongate to vermiculate or subfiliform, being also similar in color, which varies from yellowish brown to light yellow. The conidiophores, furthermore, are almost identical; these vary from 80 to 140 μ in length by 2 to 4.5 in width and are provided with septae, being yellowish brown with their extremities paler.

As regards form, color, and size of the spots produced by *Cercospora batatae,* judging from the dried Philippine specimens studied, these are

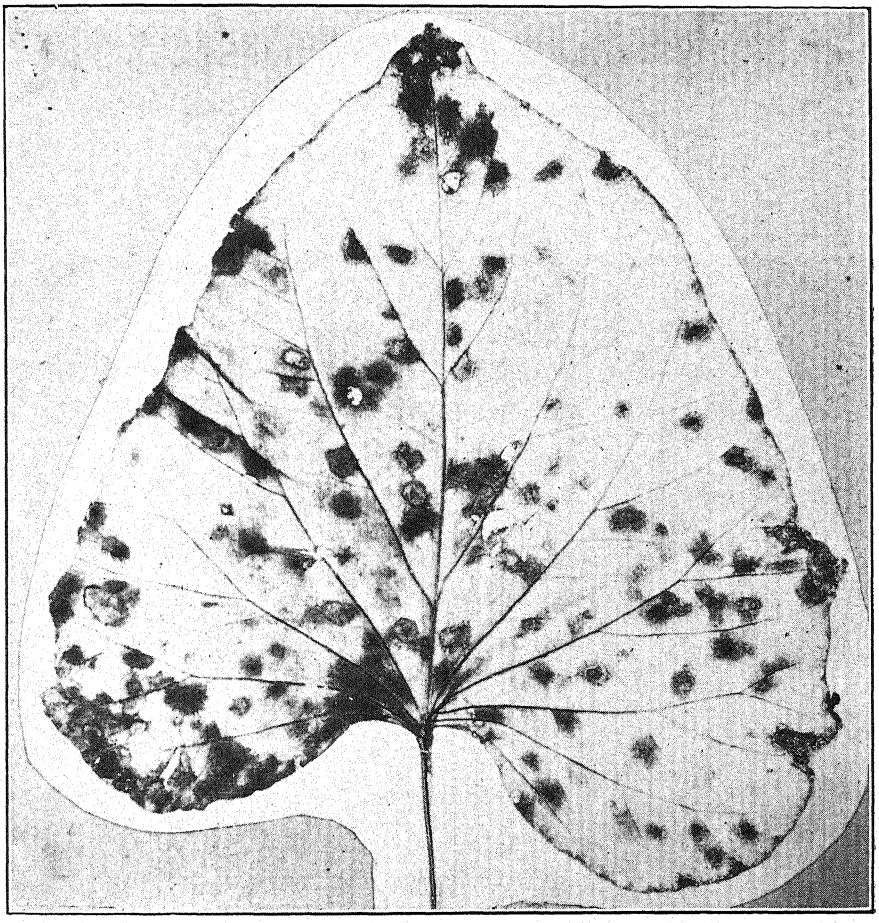


FIG. 1. Leaf of sweet potato attacked by *Cercospora bataticola,* n. sp. ×½. (Photograph by Dr. G. F. Weber.)

relatively large (usually 10 mm. or less in diameter, but sometimes as large as 15 mm.), dark, becoming almost black, irregular in shape but tending to be rounded, and usually confluent, rarely isolated. The specimens from Florida, Cuba, and Santo Domingo show smaller spots (normally 3–8 mm., rarely 2–10 mm.), isolated or subconfluent, purplish black in color, almost indefinite when young, and circular to irregular in outline (Fig. 1).

In view of these differences and in confirmation of the opinion expressed by Harter and Weimer,[3] we believe that the Cercospora found on sweet potato in America should be separated from that of the Old World as a distinct species, for which we hereby propose the name of *Cercospora batatícola,* n. sp. The reported occurrence of *C. batatae* in Brazil by Averna-Saccá[4] should be confirmed.

We are indebted to Dr. Weber for the accompanying photograph.

ESTACIÓN NACIONAL AGRONÓMICA,
MOCA, SANTO DOMINGO.
ESTACIÓN EXPERIMENTAL AGRONÓMICA,
HAVANA, CUBA.

[3] *Op. cit.*

[4] Averna-Saccá, R. *As molestias criptogamicas das plantas horticolas.* Bol. Agr. (São Paulo) 18: 382–416, 486–515, 567–583, 634–654. 1917. Quot. by Harter and Weimer.

PHYTOPATHOLOGICAL NOTE

The relation of leaf blight to sun scald of honeydew melons.—During the summer of 1929 leaf blight, caused by *Macrosporium cucumerinum* Ell. and Ev., resulted in complete drying of foliage of many honeydew melons in the Arkansas River Valley of Colorado. In some instances every plant in a field was dried and prostrate on the soil, leaving the melons exposed to the sun. After the leaves had dried, spots of varying sizes began to appear on the fruit. This spotting was observed wherever the foliage had been destroyed. Melons in fields where there was very little leaf blight were not spotted. The spots were limited in every instance to the side exposed to the afternoon sun, thus indicating that solar radiation may have been the primary cause of the conditions (Fig. 1).

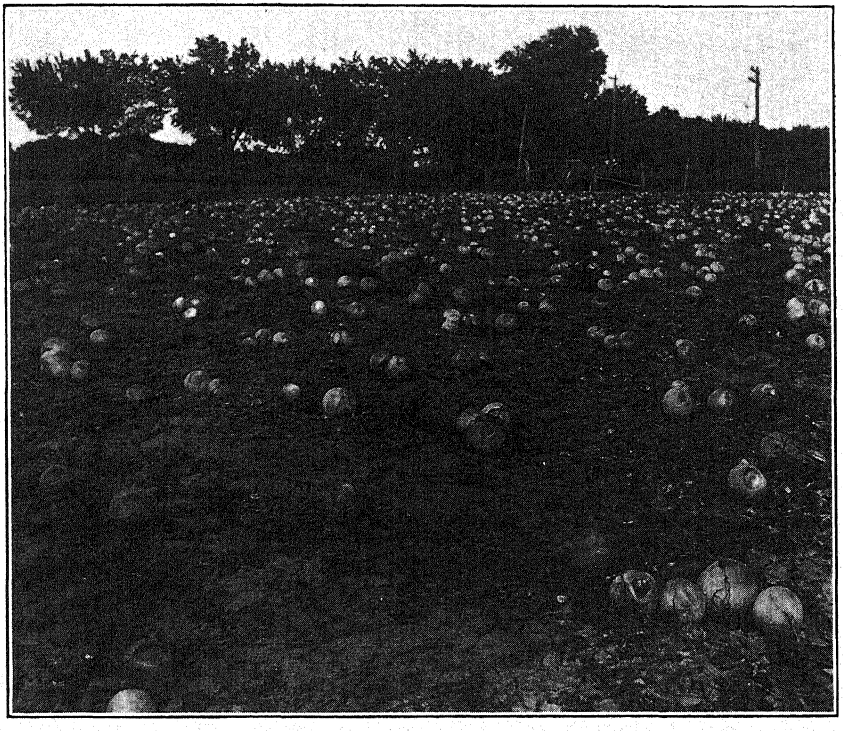


FIG. 1. Honeydew-melon field showing sun scald on the melons.

The first signs of sun scald are small brown spots upon the exposed side of the fruit. These spots enlarge slowly, as the season advances, until they attain considerable size. Spots varying from 1 inch to 5 inches in diameter were observed. The affected areas are usually round and become somewhat

sunken. They take on a dark color with age. A white to gray margin of chlorotic tissue usually surrounds the darker necrotic spots.

Not all the melons in a field were affected, the greener and less mature melons apparently being less susceptible than those that were nearly ripe. Cantaloupes and watermelons were likewise attacked with leaf blight, but these melons did not become spotted as did the honeydew melons.

Microscopic examination was made of freshly wounded tissue, but no bacteria or fungi were found. Numerous cultures were made on prune agar, potato-dextrose agar, and corn-meal agar; but no organisms developed. As the season advanced, however, and the spots became older, numerous secondary fungi and bacteria gained entrance and caused the fruit to rot. The affected fruits eventually became a soft, spongy, water-soaked mass. The fact that this condition occurred only where foliage had been destroyed and was not found where plants were unaffected with leaf blight indicates that the spots are due to sun scald. This possibility is further substantiated by the fact that the traumatic tissue is bacteriologically sterile at the onset of necrosis. It is known that ultraviolet light is present in large amounts at this elevation (3500 to 4000 feet) in the afternoon, and it is very probable that these rays are primarily responsible for the injury.

From the nature of the association of sun scald only with destruction of foliage by *Macrosporium cucumerinum*, it would seem logical to assume that any measures which would check the ravages of the leaf blight would also tend to prevent the sun scald of melons.—E. L. LECLERG, *Colorado Agricultural Experiment Station, Fort Collins, Colorado.*

BOOK REVIEW

Belling, John. The Use of the Microscope. 315 pp. McGraw-Hill Book Company, Inc.: New York, N. Y., 1930. Price $4.00.

This treatise on the microscope has appeared at a very opportune time. The attempt to keep pace with other branches of biological research has resulted in a marked increase in microscopical research and has brought about a need for just such a book as this, that shows how a microscope may be brought to its maximum efficiency.

The opening chapter gives 62 causes of injury to the microscopical image. Such an impressive list is enough to discourage all but the most optimtisic microscopist; however, in later chapters the author shows how both major and minor errors may be detected and corrected.

The following paragraphs on the light, the condenser, the object, the objectives, and the oculars show the author's method of adjusting for some of the major corrections of the high-power microscope.

He eliminates the glare from an incandescent lamp by a ground-glass filter and a diaphragm near the lamp to control the size of the light source. The intensity of the light is reduced by a light-absorbing combination of color filters. Minor reflections from the microscope mirror are eliminated by replacing it with a reflection prism.

He recommends the use of a condenser corrected for spherical and chromatic aberration; the latter correction may be neglected if use is made of the proper light filters. The condenser must be accurately centered and with these precautions the 16 mm. objective may be used with an almost full cone of light without objectionable glare.

The twin objective binocular microscope is well adapted for magnifications ranging from 10 to 100. The monobjective binocular microscope is best suited for long periods of study of objects requiring high magnifications. The object for such study is usually mounted on a glass slide and may or may not require a cover glass. All slides should be 1 mm. thick and the cover glasses should be 0.17 mm. thick to eliminate the necessity of constantly changing the correction collar of the objective or the tube length of the microscope.

The character of the mounting medium determines the proper type of objective to be used to obtain the maximum resolution and definition. The efficiency of the microscope centers around the objectives. They should be optically clean and accurately centered in the light axis. For low-power studies the 16 and 8 mm. apochromatic objectives are well adapted. The useful magnification of an objective ranges from 530 to 1060 times the working aperture and it is advisable to keep within this range. Water- and

oil-immersion lenses, depending on the character of the preparation, are often to be preferred to the high-power dry lenses.

The author considers the draw tube almost a nonessential feature of the microscope if slides and cover glasses of the proper thickness are used and the higher dry and water-immersion lenses are equipped with correction collars. All binocular microscopes, however, should have some way of preventing a change in tube length that occurs in most makes when the interocular distance is changed. It is infrequently necessary to make corrections by means of the draw tube when objects at different depths in the mounting medium are being studied.

Oculars of the compensating type, when light corrections are made by filters for achromatic objectives, are recommended by the author for general use, except in photography, where special oculars giving a flat field are essential. Oculars with a low magnification give a curved field and should be avoided by changing to a lower objective.

The author has used with great success water-immersion lenses. They are superior for material in a water medium if the object is more than 10 microns below the cover glass. They are, however, primarily for the skilled microscopist, owing to the accurate corrections that must be made with the collar if maximum resolution and definition are to be obtained. The reviewer does not see why microscopists need to make a hasty change from the highly perfected oil-immersion lenses to water immersions so much used by the author even for aceto-carmine preparations. It is customary to use only a thin layer of aceto-carmine and the pollen mother cells are normally in contact with both the cover glass and slide. With objects so prepared the major reason for using a water-immersion lens that requires accurate adjustment for each preparation is obviated.

It seems that many of the author's suggestions would have been more convincing if the optical reasons for the changes had been given. Perhaps the author has spared us by omitting complicated formulae which underlie his suggestions for improvement; however, the reviewer hopes that some capable critic will show that the author's suggestions are based on accepted optical theory.

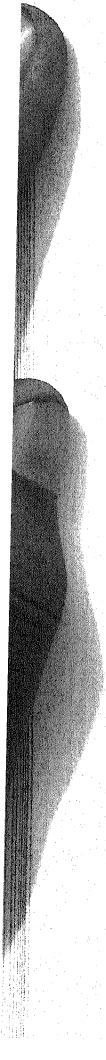
Testing the microscope and adjusting it to its maximum efficiency have been made possible if the microscopist follows the instructious outlined in the chapters on tests and rules for high-power and routine microscopy. The star test detects axial spherical aberration, inaccurate tube length, and improper adjustment of the correction collar. With everything correct the disc or ring into which the small spot of light expands, when out of focus of the microscope, will be equally sharp at equal distances within and without the focus, though it may not be equally bright. The ring test is used

to correct the condenser. If properly focused the light of the aperture will be greatest and solid. Raising or lowering the condenser, only, reduces the aperture without giving marginal rings of light.

Doctor Belling has prepared an excellent glossary and index. The text is full of suggestions to help the student of the microscope acquire efficiency in its manipulation and at the same time reduce the fatigue that often comes from constant looking through hand lenses, twin objective binoculars, and high-power microscopes. There are many departures from the usual practice of slide preparation, adjustment of light and lenses, and making camera-lucida drawings and photomicrographs, which years of experience have shown to be practical.

Instructors and professors of biology in many of our universities will go through the next few semesters with a worried look. Their students will remind them that one of our leading microscopists tips his microscope at an angle of 45°. The use of a microscope stage tipped at an angle of 45° might prove very unsatisfactory for studying material mounted in an aqueous solution with immersion lenses if it allows the objects and the immersion liquid to settle to the lower side of the slide. Two of the leading microscope manufacturers, however, are producing microscopes with a horizontal stage and oculars tipped at an angle of 45°, thus eliminating the objectionable feature of tipping the microscope stage and the strain from bending over a microscope in the vertical position.

The general use of aceto-carmine is due to Doctor Belling's demonstration of its applicability as a chromosome stain. Each individual user likely has found some modification to make this killing and staining solution more applicable to his material. So it seems with these usfeul hints on microscope manipulation, microscopists will begin at once to eliminate some or many of their errors, but each worker is likely to find some modification of the rules to suit his particular need.—A. E. LONGLEY, *Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.*



ABSTRACTS OF PAPERS PRESENTED AT THE TWENTY-SECOND ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, CLEVELAND, OHIO, DECEMBER 30, 1930, TO JANUARY 1, 1931, INCLUSIVE

Effect of solid carbon dioxide upon transportation diseases. CHARLES BROOKS.

Solid carbon dioxide now furnishes a convenient means of increasing the carbon-dioxide content of storage air and has been used experimentally for this purpose in small containers, in pony refrigerators, and in standard refrigerator cars. Within a half-hour to an hour after a car is loaded it is possible to increase the carbon-dioxide content of the atmosphere to a percentage that will have a checking effect on the rotting and softening of warm fruit equivalent to a 30 to 40 degree F. drop in temperature. If the gas escapes within the next 18 to 24 hours no objectionable flavor is likely to result, but with peaches, strawberries, and red raspberries there is the possibility of a reduction in flavor and with more extreme treatments the fruit may become quite flat and insipid. Dewberries, blackberries, plums, and cherries are more resistant to carbon-dioxide injury. Grapes, sweet corn, peas, and beans seem to offer the greatest promise of beneficial effects without harm to the product. The inhibiting action of the gas ceases soon after restoration of normal atmospheres, but by this time the car has been fairly well cooled.

Further studies with psyllid yellows of the potato. B. L. RICHARDS.

In repeated tests the adult form of *Paratrioza cockerelli* in numbers up to 1,000 per plant has failed to produce symptoms of psyllid yellows on the potato plant. Nymphs from adults used in such tests, as well as from all other sources employed, when used in sufficient numbers, produced the disease uniformly. All attempts to date to separate nymphs from the infective principle by growing young nymphs on healthy plants from eggs hatched on healthy leaves in petri dishes have failed. The type of symptoms and the degree of injury to the potato appear to be definitely correlated with number of nymphs feeding, length of feeding period, and the intensity and duration of light exposure. Under greenhouse conditions psyllid yellows is not induced uniformly with fewer than 15 nymphs. With larger numbers, symptoms appear in from 4 to 6 days. The progress of the disease is interrupted, and the plant apparently may assume a normal expression, if the feeding nymphs are removed from the infested plant in 5 to 10 days after appearance of the first symptoms. Growth, which is stimulated by insect feeding, but which occurs subsequent to their removal, is to all appearances normal. In Utah, normal plants are obtained from tubers grown on psyllid-infested plants.

Aphids as vectors of leaf roll among sprouting potato tubers. F. C. STEWART and H. GLASGOW.

In June, 1928, and again in March, 1930, potatoes purchased at grocery stores in Geneva, N. Y., were found infested by large numbers of the spinach aphid, *Myzus persicae*. The occurrence of aphids on sprouting potato tubers is frequent in England and Ireland, but has not been previously reported from America. Experiments conducted by the writers show that the aphids may spread leaf roll among sprouting tubers.

In one experiment halves of 38 tubers from healthy plants were mixed with aphid-infested leaf-roll tubers and allowed to sprout for 20 days. The aphids multiplied and

spread to the sprouts of the healthy tubers. Each tuber-half was then cut crosswise into two pieces and all planted in pots in a greenhouse. Of the 76 plants produced 5 appeared normal, 68 showed positive symptoms of leaf roll, and 3 were doubtful. Under parallel conditions the other halves of the same tubers used as a check and not exposed to aphids produced 76 normal plants.

In another experiment it was proved that normal and leaf-roll plants may be obtained from different sprouts of the same tuber by allowing the aphids to feed upon some sprouts and excluding them from others.

The distribution of the latent virus in tubers of commercial potatoes. GROVER BURNETT and LEON K. JONES.

Tomato plants were inoculated with macerated leaf tissue of plants grown from commercial potatoes combined with macerated leaf tissue of tobacco plants affected with common tobacco mosaic. Apparently healthy plants from 93 commercial tubers were used in the tests with the following varieties represented: Netted Gem, Early Rose, White Rose, Wisconsin Pride, Burbank, and Beauty of Hebron.

One tuber of the Early Rose variety was found to be free from the latent virus. During the growing season in the field the foliage of four plants produced from this tuber remained free from the latent virus, while foliage of another plant from this tuber, after being inoculated with macerated tissue of a potato affected with rugose mosaic, produced streak in tomato when leaf tissue from it was inoculated in combination with tobacco mosaic.

The use of disinfectants in fertilizers for the control of potato scab and rhizoctonia. WM. H. MARTIN.

During the past 5 years a series of field and greenhouse experiments have been conducted to determine the value of the addition of various disinfectants to fertilizers for the control of soil-borne scab and rhizoctonia of the potato. The materials used included calomel, yellow oxide of mercury, formaldehyde, and various organic mercury compounds. These materials were mixed with the fertilizer and the resulting mixture was then applied in the open furrow in the usual manner, before planting the crop. Except where they were improperly incorporated in the soil, the potatoes developed normally and an appreciable reduction in scab and rhizoctonia followed. In an experiment in 1930 the following results were secured:

Treatment	Yield per acre	Free from scab	Severe rhizoctonia
	Bushels	Per cent	Per cent
Fertilizer alone	143.3	65.8	51.7
Fertilizer and 20 lbs. calomel per acre	176.0	93.9	2.7
Fertilizer and 10 lbs. yellow oxide of mercury per acre	147.9	97.4	3.1
Fertilizer and 45 lbs. Semesan per acre	170.7	93.7	1.8

Similar results have been secured in other experiments with potatoes. The scope of this work has been broadened to determine the value of this treatment in the control of soil-borne pathogenes attacking certain vegetables and ornamentals.

The effect of depth of planting and soil moisture on the development of rhizoctonia on the potato. E. S. CLARK and WM. H. MARTIN.

Cankering of the stems and stolons of the potato was much more severe in soils adjusted to 20 per cent moisture than in those with higher moisture content. The number of sclerotial bodies was likewise greater in the low- than in the high-moisture series. The disease was more prevalent on sprouts from seed pieces planted 3½ inches deep than on the sprouts of those planted 1½ inches. The severity of the disease at different planting depths was also influenced by soil-moisture content. Sprouts from seed pieces planted 1½ inches deep in a soil maintained at 20 per cent moisture showed more serious infection than those planted 3½ inches deep in a soil adjusted to a moisture content of 60 per cent. In general, however, deeper planting influenced the development of rhizoctonia in the same manner as a deficiency in soil moisture.

The effect of different pressures and of different types of lime in potato spraying. PAUL E. TILFORD.

In 1929 the increases due to spraying potatoes with 4–6–50 Bordeaux mixture were significantly greater when the spray was applied at 400 pounds pressure than at 200 or 600 pounds. In 1930, when there was very little rainfall to remove the spray, Bordeaux applied at 200, 400, and 600 pounds pressure gave practically the same results.

Experiments over a period of 4 years, comparing the increases obtained from spraying with Bordeaux prepared from stone lime, and from high calcium-hydrated lime, show hydrated lime to be as satisfactory as stone lime. Tests conducted by growers in commercial fields gave as good results from hydrated lime as from stone lime.

Bordeaux, prepared from high calcium-hydrated lime, gave greater increases than that prepared from high magnesium-hydrated lime.

The benefits from spraying in these experiments have been largely due to control of hopper burn and protection from the heat.

Seed treatment for damping-off of tomatoes. JAMES G. HORSFALL.

A chemical seed treatment of tomatoes for damping-off in seedling flats has been evolved for growers who have found soil steaming undesirable. Copper fungicides, notably 5 per cent $CuSO_4$ as a soak for 1 hour or as monohydrated dust and $CuCO_3$ dust, in 4 duplicate trials with naturally contaminated soil, increased emergence 205.3, 228.4, and 135.7 per cent and reduced disease in emerged seedlings to 20.0, 30.4, and 63.4 per cent of the check, respectively. In two tests the following additional copper compounds as dusts increased stands and decreased disease; cupric oxalate, tartrate, sulphide, silicate, arsenate, oxide, chloride, cuprous chloride, bromide, sulphide, thiocyanate, copper oxychloride, and malachite. Except for a slight lag, tomato seeds grew normally after soaking in an 8.6 per cent $CuSO_4$ solution for 24 hours or in a 50 per cent for 2 hours. Mercury compounds gave good disease control but injured germination under certain ill-defined conditions. Semesan, for example, reduced disease to 15.5 per cent of the check in 10 duplicate trials, but reduced emergence in one case to 7 per cent or incresed it in another to 171 per cent of the check. The average emergence, however, was 112.6 per cent of the check.

Effect of seed treatments on seed longevity. E. E. CLAYTON.

Seeds of many vegetable crops are often stored for 2 to 5 years before sowing. The use of recommended methods of seed treatment is greatly restricted by fear of seed injury. Results from six years' experiments with many kinds of seed show that this fear

is well founded. Recommended treatments with (1) mercuric chloride, (2) liquid organic mercurials, and (3) hot water have greatly shortened the life of the seed, even when they caused no immediately apparent injury. The dust treatments usually had no injurious after effects. Experimental work shows that much can be done to reduce or eliminate the harmful after effects of seed treatment. For example, with three lots of cabbage seed the germination 18 months after treating was:—

25 minutes at 50 degrees C. in water	51.60%
" " " " " " " .25% $ZnSO_4$	62.21%
Nontreated	53.30%

Other promising possibilities are indicated. The effective use of seed treatment requires close cooperation between seed producers, seed dealers, and the crop growers. An instance in which such cooperation has been successfully worked out with cauliflower seed is briefly discussed.

The growth rate of tomato plants affected by yellows. MICHAEL SHAPOVALOV.

The rate of elongation of tomato stems of both inoculated and uninoculated plants was ascertained by measurements made at different intervals. Three different types of curves, representing three different types of growth, were obtained. Healthy plants, whether inoculated or not, give practically the same, gradually rising curve (I). Inoculated plants which developed the disease and never recovered from it show at first a slight lag, then a sudden cessation of growth (II). Plants affected in the beginning, but later showing signs of recovery, are apt to lag at first, then show a rapid rise in the rate of growth, followed by a gradual increase, as in checks (III). Shading does not change the types of curves but reduces the number of positive cases and thus increases the number of plants giving (I) and (II) types of curves and speeds up the rate of elongation. In early stages of tomato yellows a slight decrease in the rate of growth of diseased as compared with healthy plants often precedes the appearance of other externally recognizable symptoms. The lag and the cessation of growth in these cases are accompanied by a rapid accumulation of carbohydrates and an increase in total nitrogen and, therefore, do not signify the weakening of either the photosynthesis or the absorption of nutrient elements from the soil. All these phenomena, however, suggest a serious disruption of the metabolic processes.

The occurrence of the Australian spotted wilt of tomatoes in Wisconsin. S. P. DOOLITTLE and C. B. SUMNER.

There appeared in the field at Madison, Wis., during the summer of 1930 a virus disease of tomatoes which seems identical with the so-called spotted wilt of tomatoes in Australia. The disease is a form of streak and the affected plants showed symptoms apparently identical with those described from Australia in a recent paper by Samuel, Bald, and Pittman. The young leaves developed the peculiar bronze markings typical of the Australian disease, the petioles and stem also being affected to an extent which occasionally resulted in the death of young plants. Fruits also were found showing the peculiar concentric markings figured in the Australian paper. The disease appeared on occasional plants during the season but did not spread rapidly in the field. It is readily transmitted to tomato by artificial inoculation but no work has yet been done on insect transmission. Its relationship to other forms of streak has not been determined.

Hybridization between Puccinia graminis tritici and Puccinia graminis secalis. MARGARET NEWTON, T. JOHNSON, and A. M. BROWN.

Crosses between forms 30 and 95 of *Puccinia graminis tritici* and a field culture of *Puccinia graminis secalis* have resulted in the production of four hybrid rust forms,

three of which have not hitherto been described. These hybrids, although distinguishable, resemble each other rather closely in pathogenic characters. They are much less virulent on wheat varieties than other known physiologic forms of the *tritici* series and are likewise less virulent on rye varieties than forms of the *secalis* series. If these hybrids are characteristic of all hybrids between the *secalis* and *tritici* varieties of *Puccinia graminis*, it is probable that such hybridization is not important as a source of virulent strains of wheat stem rust or rye stem rust.

A synthetic production of Puccinia graminis hordei F. and J.—MOSES N. LEVINE and RALPH U. COTTER.

By crossing pycnia of *P. graminis secalis* and *P. graminis tritici* on barberry, the writers recently obtained a strain of stem rust that attacks certain varieties of rye and wheat rather severely, which neither of the parents alone can do. Barley also is quite susceptible to this rust hybrid. These results indicate the production through hybridization of a rust form, not only pathogenically different from either one of the parents but possessing to a certain extent the parasitic properties of both. The actual existence of *P. graminis hordei* may here be involved. (Cooperative investigations between the Offices of Cereal Crops and Diseases and Barberry Eradication, Bureau of Plant Industry, United States Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Nuclear association in the aecium of Puccinia graminis. W. F. HANNA.

The sporidia of *Puccinia graminis* are uninucleate. Under certain conditions they have been found to produce secondary sporidia. Monosporidial infections on the barberry give rise, on the upper surface of the leaf, to pycnia containing many uninucleate pycniospores and, near the lower surface, to crescent-shape wefts of uninucleate hyphae. The stimulus of gravity does not appear to determine the particular area of the leaf in which these organs develop. About forty-eight hours after pycniospore-containing nectar from a pycnium of one sex is applied to a pycnium of opposite sex, binucleate cells appear in the hyphal wefts near the lower surface of the leaf. Shortly afterwards, multinucleate cells are found near the center of the weft; as many as sixteen nuclei have been counted in a single cell. Some of these binucleate and multinucleate cells arise by cell fusions. The paired nuclei of the aeciospores are considered to be descendants of nuclei which become associated in this manner. In one preparation a nucleus was found which appeared to be "migrating" through the cell wall into a neighboring cell. A few germinating pycniospores have been found, but their rôle in initiating aecial development is not fully understood.

Indications of heterothallism in Tilletia tritici. H. H. FLOR.

In a series of greenhouse and field tests wheat seedlings were inoculated with one or more cultures from single primary sporidia of *Tilletia tritici.* Ten such monosporidial cultures inoculated singly into wheat seedlings produced no infection. Of six trials in which the seedlings were inoculated with a mixture of two such monosporidial cultures, infection was obtained in three. Three trials in which ten of the monosporidial cultures were mixed produced infection in every case. In no instance were uninoculated plants infected. (Cooperative investigations by the Washington Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.)

Physiologic forms of bunt of wheat and varietal resistance. E. N. BRESSMAN.

Studies were made over a three-year period of the parasitic behavior of 100 collections of bunt, *Tilletia tritici* and *T. levis*, obtained from widely separated sections of the United States and several foreign countries. Ten standard varieties, nine reported as resistant and one as susceptible to bunt, were used as differential hosts and subjected to the usual inoculation tests. The results indicated that there are at least 10 physiologic forms of bunt, six *T. levis* and four *T. tritici*. Practically all of the varieties of wheat, formerly classed as resistant to this disease, are susceptible to one or more of these forms. White Odessa, Martin, Banner Berkeley, Albit, Regal, Sherman, Stephentshka, Cooperatorka, Hope, and Hussar are susceptible to several forms. Ridit, although resistant to most forms and collections, is susceptible to some forms. Turkey × Bd. Minn. No. 48, Hohenheimer "behaart" and Hohenheimer "unbehaart" are rather resistant to most of the forms but some infection is obtained. Rye was susceptible to several of the collections. *T. secalis* is designated as a form of *T. tritici*. Hosar, a selection by the writer, from Woolman's hybrid, Hussar × Hohenheimer "behaart" has been consistently resistant to all of the available collections of bunt.

Germination of wheat stem-rust teliospores formed in the greenhouse. THORVALDUR JOHNSON.

Attempts were made by freezing and by alternate wetting and drying to shorten the dormancy period of teliospores formed in the greenhouse. Either of these treatments, in most cases, reduced the period of dormancy. A combination of the two, that is, a short period of freezing (2–7 days) followed by alternate wetting and drying, invariably resulted in germination. Teliospores formed at low temperatures, 55°–60° F., germinated more abundantly when subjected to this treatment than those formed at higher temperatures, 70°–75° F.

Teliospores formed in nature on *Hordeum jubatum* and *Agropyron repens* in September, 1929, likewise responded to freezing followed by alternate wetting and drying and germinated abundantly at the beginning of December. Teliospores on *Hordeum jubatum* and wheat collected out-of-doors, September 3, 1930, commenced germinating September 19, after being subjected to the same treatment.

As to the minimal dormancy period of teliospores produced in the greenhouse, the shortest period between the completion of teliospore formation and the germination of the spores was 20 days. Spores frequently germinated between 30 and 40 days after formation. The shortest period from the inoculation of wheat plants with urediniospores to the germination of the teliospores formed on them was 55 days.

Effect of mineral nutrition on the reaction of wheat varieties to leaf rust. K. D. DOAK.

Wheat varieties showing various types of reaction to one physiologic form of leaf rust, *Puccinia triticina*, were grown in sand cultures providing different degrees of excess and deficiency in nitrogen, phosphorus, and potassium. Nitrogen increased susceptibility, while phosphorus and potassium decreased it. Excess nitrogen induced the development of larger primary uredinia and more abundant secondary uredinia and decreased chlorosis. Phosphorus in excess increased chlorosis, did not reduce the size of primary uredinia, and retarded or prevented development of secondary uredinia. Excess potassium increased chlorosis and also decreased the size of primary uredinia. Secondary uredinia appeared in incomplete rings around the primary. In varieties of intermediate reaction, excess nitrogen increased the percentage of infected points with uredinia, while nitrogen deficiency, excess phosphorus, and excess potassium decreased it. Phos-

phorus deficiency decreased chlorosis in both susceptible and intermediate varieties, but the uredinia were always small. Potassium deficiency decreased chlorosis but did not reduce the size of uredinia. A few resistant varieties were affected like the intermediates. The relation of time of starvation and excess to change in reaction was tested in water cultures.

The effect of temperature and light on the development of the uredinial stage of Puccinia graminis. LEONARD W. MELANDER.

Urediniospores of *Puccinia graminis*, hardened ten days by exposure to 0 to 1° C., withstood low temperatures on dry glass slides in a freezing chamber better than non-hardened spores. Hardened urediniospores of wheat, timothy, and oat stem rust survived at -29° C. to -40° C. for 40 to 45 days. After six days of alternating high and low temperatures the percentage of viable, hardened spores was lowered.

At 10° C. uredinia appeared a week later than at 20° C. They developed slowly at 0 to 1° C., and sometimes the infection type differed from the normal type produced at 10° and 20° C. *P. graminis tritici* form 36 produced numerous uredinia after 59 days at 0 to 1° C.; form 35 produced minute uredinia on Little Club wheat; form 15 generally failed to produce uredinia within 80 days. When reexposed to 20° C., type-1 uredinia became type 3; and form 15, dormant at 0 to 1° C. in wheat plants, produced normal uredinia.

Production of telia on wheat, oats, and rye was stimulated at 0 to 1° C. Both rust and host withstood – 10° C. for 24 hours.

Low light intensities retarded stem-rust development. Urediniospores of *P. graminis tritici* form 15 formed in a light intensity of 302 foot-candles at 20° C. were longer and better able to withstand temperatures of – 29° C. to – 40° C. for 24 hours than spores formed in higher or lower light intensities. (Cooperative investigations between the Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Relationship of the oat smuts. W. F. HANNA and W. POPP.

Experiments in which oat seedlings inoculated with cultures of *Ustilago avenae* and *U. levis* were grown to maturity in the greenhouse yielded the following results: (1) Plants inoculated with a single monosporidial culture of *U. avenae* or *U. levis* did not produce smutted heads. (2) Plants inoculated with two monosporidial cultures of opposite sex produced smutted heads. If the two cultures were of *U. avenae* the infected heads were "loose" in appearance and their spores echinulate; if of *U. levis* the heads were "covered" in appearance and their spores smooth; if one of the cultures was of *U. avenae* and the other of *U. levis* the infected heads were somewhat variable in appearance, but upon close examination they proved to be of the loose type, and their spores were echinulate. (3) The sporidia of *U. avenae*, like those of *U. levis*, are of two kinds, (+) and (–); the sporidia of one species mate without difficulty with sexually opposite sporidia of the other species.

These results indicate that *U. avenae* and *U. levis* are genetically distinct with respect to the characters by which they are differentiated, but the ease with which crosses can be made between them suggests that they are closely related species.

Host specialization and parasitism of the genus Rhynchosporium. RALPH M. CALDWELL.

Results of additional experiments have now been secured with monosporous cultures of the scald fungus, *Rhynchosporium secalis* (Oud.) Davis from *Agropyron repens, Bromus inermis, Dactylis glomerata, Elymus robustus,* barley, and rye collected in

different localities. Two to five isolations from each host have been tested. The hosts inoculated included rye, barley, oats, wheat, *Danthonia californica*, *Hordeum jubatum*, and *Lolium perenne*. No culture infected any host other than that from which it came. In these cases significant infection almost invariably occurred. There is evidence of five host-specialized races of *R. secalis*. Excepting the scald fungus on Dactylis, no significant morphological variations were found within the species *R. secalis* as it occurred upon several hosts. However, upon *Dactylis glomerata*, the fungus is clearly distinct in morphology and parasitism. It is being described as a new species. The two species develop on the hosts in a similar and unusual manner. Penetration is direct from appressoria of conidial germ tubes. The fungus body consists primarily of a subcuticular stroma with very limited and delayed growth of hyphae in the mesophyll.

The development of crown gall, hairy root, and callus under controlled conditions. A. J. RIKER, W. M. BANFIELD, and G. W. KEITT.

Crown gall, caused by *Phytomonas tumefaciens;* hairy root, caused by *Phytomonas rhizogenes;* and wound overgrowth, caused by girdling, were each produced under conditions designed to avoid the presence of complicating factors. This was done by inducing these enlargements (1) on nursery apple trees grown in steamed soil both in the field and in the greenhouse and (2) in the field on stems of nursery apple trees, the treated parts of which were enclosed in small chambers designed to permit a disinfectant treatment and to prevent foreign organisms from gaining entrance. Cultures of single-cell origin and tested pathogenicity were employed.

After one and two years the results showed that inoculations with the crown-gall organism induced only crown gall, those with the hairy-root organism induced only hairy root, and girdling induced only callus and wound overgrowth. Mixed cultures of the crown-gall and hairy-root organisms caused malformations which showed complete intergradations of crown-gall and hairy-root characters. The pathogenic organisms gained entrance into the host plants readily through wounds, but callus appeared not ordinarily to be an open infection court for either of these bacteria. (Cooperative investigations between the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station.)

The life cycle of the hairy-root organism on apple in relation to pathogenesis. E. M. HILDEBRAND.

The life cycle of *Phytomonas rhizogenes*, the hairy-root organism, is being studied in relation to pathogenesis on nursery apple trees.

The entrance of the bacteria into the host tissues is accomplished only through wounds which heal so rapidly that the bacteria are unable to enter a wound three days old. The callus tissue which develops following a wound was determined not to be commonly an open infection court. Different kinds of insects were observed to be feeding on the callus, roots, and diseased tissue below ground. Efforts to isolate crown-gall and hairy-root bacteria from these insects have been unsuccessful.

The exit of the bacteria from the overgrowths seems to be a continuous process from the surface, where the organisms commonly occur in abundance. As the enlargements become older they contain correspondingly more xylem elements, from which the bacteria are less easily isolated. These bacteria may overwinter in the soil and may exist there separated from the host for over a year. (Cooperative investigations between the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station.)

Physiologic specialization in Gymnosporangium. DONALD E. BLISS.

Statements from various parts of the United States are much at variance regarding the relative susceptibility of certain varieties of apple, *Pyrus malus*, to *Gymnosporangium juniperi-virginianae* and *G. globosum*.

Collections of *G. juniperi-virginianae* from Iowa, Kansas, and Wisconsin, when placed on the Tolman and York Imperial varieties, caused only flecking. However, *G. juniperi-virginianae* from West Virginia produced aecidial cups on the same hosts. Bechtel's Double-flowering crab, *Pyrus ioensis*, was highly susceptible to all of these collections. Varieties of apple trees secured from nurseries in Iowa, Kansas, New York, and Virginia were alike in their reactions. These data are indicative of physiologic specialization in Gymnosporangium.

Further inoculations were made, using three collections of *G. globosum* from Iowa. All of these caused an abundance of aecidia to form on *Crataegus mollis*, but flecking was the only symptom to develop on the Fameuse, Tolman, Yellow Transparent, York Imperial, Wealthy, McIntosh, Baldwin, Delicious, and Northwestern Greening varieties. Although the aecidia of *G. globosum* were found commonly in Iowa on *Crataegus* spp., none were observed on the leaves of 150 varieties of apples examined in 1930. *G. globosum* does not seem to occur on apples grown in the nursery in Iowa, although it has been identified on many varieties in New York.

Pathogenicity of three red cedar rusts that occur on apple. P. R. MILLER.

Successful inoculation was obtained with *Gymnosporangium germinale*, quince rust, on the fruit of Delicious, Winesap, Stayman, and Wealthy apple varieties and on quince foliage; with *Gymnosporangium juniperi-virginianae*, apple rust, on the fruit and foliage of Rome, Ben Davis, Grimes, and Wealthy and on the foliage of Jonathan; and with *Gymnosporangium globosum*, hawthorn rust, on the foliage of Maiden Blush, Rome, Ben Davis, Jonathan, Grimes, and Wealthy, and in addition on pear and *Sorbus* sp. These results are more or less in accord with the natural infection as observed in 1929. Apple fruits were susceptible for approximately fifteen days after petal fall. Infection was obtained through wounds on apple leaves which had become resistant. Leaves removed from the tree at the end of the day remained alive two months on a sucrose solution in Petri dishes. Indications of heterothallism were secured by mixing the pycnial exudates. Overwintering of aeciospores resulted in a marked increase in the percentage of germination. On an awn-leaf type of red cedar large galls of *Gymnosporangium juniperi-virginianae* were found on large limbs.

Brooks fruit spot. H. C. YOUNG and FRANK WINTER.

During the season of 1928 "fruit spot" caused a severe loss in the apple crop in the Ohio valley. Previous to this nominal losses occurred in widely separated orchards in southern Ohio but no general epidemic had occurred. Consequently, not much attention had been given to control measures.

A detailed study of the causal fungus, *Mycosphaerella pomi*, was begun in the spring of 1927. It was found that the perfect stage of the fungus occurs on almost all types of deciduous leaves throughout the State. The ascospores mature earlier in northern than in southern Ohio.

The humidity, temperature, and host relationships of the fungus are extremely interesting. The optimum temperature for growth is 22° to 24° C. Growth was almost stopped at 30° C.

It was found that dilute Bordeaux mixture (1–3–50) controls the fungus. During the season of 1929 the 4-weeks spray was the most effective. On account of the drouth no results were obtained in 1930.

Cultural characters and host range of the apple sooty-blotch fungus. M. W. GARDNER and R. C. BAINES.

By partial surface sterilization pure cultures of the sooty-blotch fungus, *Gloeodes pomigena,* have been obtained from apple fruits. The fungus grows very slowly and on potato agar produces a thick black thallus on and around which gelatinous masses of spores accumulate. The fungus grows well on a variety of media but has sporulated only on potato, malt-extract, and prune agars. On very moist agar the spores bud profusely. Cultures similar to those from apple have been obtained from sooty blotch on the young wood of leatherwood, *Smilax hispida,* prickly ash, pawpaw, *Vitis cordifolia,* white ash, tulip tree, hard maple, willow, hawthorn, blackberry, sassafras, redbud, bladdernut, *Evonymus americana,* and *Cornus rugosa* and from fly speck on the first ten species above mentioned and sycamore, spice bush, and *Cornus alternifolia.* Sooty blotch has been produced on apples by inoculation with spore suspensions from cultures from sooty blotch on apple, pawpaw, and Crataegus and fly speck on white ash and sycamore. The incubation period in a cool moist compartment in the greenhouse was about three weeks and, in the orchard, much longer.

Artificial infection of fruit with the apple-scab fungus. C. O. BRATLEY.

The length of the period of susceptibility of apple fruit to infection by *Venturia inaequalis* is important because of its relation to the value of late-summer applications of fungicides and to the appearance of new lesions on the fruit in storage.

Inoculations during the summer of 1930 were made by rubbing conidia from infected leaves directly on the moistened surface of attached McIntosh fruit. The inoculated apples were immediately inclosed in small moist chambers made of wire netting covered with wet cotton, the whole then being inclosed in bags made of transparent moisture-proof cellophane.

When continuous wetting was maintained for 40 or more hours the inoculations were usually successful but when the fruits were dry at the end of 28 hours they failed. Inoculations made during August indicated that the length of the incubation period varied from one to two months, depending on the length of the moist period at inoculation. Infections resulting from inoculations made the last of August appeared six weeks later, three weeks after the apples had been picked and placed in storage.

Bagging experiments conducted on the same variety of apples showed that natural infection occurred as late as August 13.

The relation of root-feeding arthropods to crown-gall infection on raspberry. W. M. BANFIELD.

Seasonal-development studies of crown gall on red raspberry, grown in inoculated field soil during 1928 and 1929, show that (1) 92 per cent of plants became infected in the first season, averaging 5.8 infections per plant. (2) Infection appeared to occur at random on all underground parts and did not seem to be correlated with particular stages of development of these parts. (3) Infection was most abundant on roots and stems in the upper four inches of soil. (4) Infection commonly occurred through arthropod injuries.

No crown gall occurred on 118 plants during 1929 and 1930 in inoculated soil from which root-feeding arthropods were excluded except at points mechanically injured. Crown gall occurred on 63 of 76 check plants grown without arthropod exclusion, averaging 5.2 galls per diseased plant.

White grubs, *Phyllaphaga* spp., were found to be the dominant root feeding-form present in raspberry plots in August, 1930. The injuries found on plants in these plots,

through which infection commonly occurred, appeared to be due to grubs. In pot and cage experiments, using inoculated soil, grubs caused injury to raspberry roots identical to that found in the plots. Crown gall commonly developed at these grub injuries.

Crimp, a nematode disease of strawberries. A. N. BROOKS.

Crimp, also known as French bud, briar bud, etc., has been present in Florida for many years. It probably is identical with dwarf plant of Louisiana and red plant of England. Apparently, crimp is more destructive in Florida than elsewhere.

Crimp is fundamentally a bud disease affecting the development of young leaves. Affected leaves range in size from mere rudiments to almost normal, are crimped, darker green, and more brittle than normal, less pubescent, and usually reddish in places.

Nematodes, *Aphelenchus fragariae*, most numerous, were found abundant in diseased buds, *e.g.*, 7,597 nemas in 31 buds, and practically none in healthy buds, 2 nemas in 30 buds. These nemas were found to be ectoparasitic, not endoparasitic.

Uniformly successful inoculations were made by introducing suspensions of the nemas into buds of healthy plants. Hypodermic-needle inoculations with the filtered extract of diseased plants did not produce crimp in healthy plants. Strawberry aphids and red spider did not transmit the disease from crimped to healthy plants.

Warm water treatment of crimped plants, 48° C. for 20 minutes, killed the nemas in the buds and restored the plants to normal growth.

Control consists of setting clean plants in clean soil and roguing.

Anthracnose of strawberry caused by **Colletrotrichum fragariae**, *nov. sp.* A. N. BROOKS.

Anthracnose, a spot disease occurring chiefly on strawberry runners, and occasionally on petioles, has been observed in central Florida for the past four years. The disease appears at its worst during the summer rainy season. The chief damage results from the girdling of runners before the young tip plants have put out roots and become self-supporting. Some fields have shown a 50–75 per cent kill of young plants. The average for the entire area is 2–5 per cent.

The mature diseased spots are from one to several centimeters long, girdling the runners, sunken, brown to black in color, the line of demarcation between the healthy and diseased tissue being quite sharp. Under a strong hand lens tufts of setae can be observed in profile in the spots.

The disease has been proved to be caused by a species of Colletotrichum, for which, since it appears different from other described species, the binomial **Colletotrichum fragariae**, nov. sp., is suggested.

Spraying with Bordeaux mixture 4–4–50 at ten-day intervals has been found greatly to reduce the amount of the disease but, because of frequent rains, does not completely control it.

Melons resistant to powdery mildew. IVAN C. JAGGER and G. W. SCOTT.

Powdery mildew (*Erysiphe cichoracearum* DeC. ?) first appeared in destructive form in the extensive melon, *Cucumis melo*, fields of Imperial Valley, California in 1925 and has been more or less injurious every season since, causing particularly heavy commercial losses in 1926 and 1930. Since 1926 a large number of melon varieties have been tested each season. During the first 2 years only limited resistance was found, but in 1928 mixed lots of seed from India gave numerous plants which were almost immune from mildew. These melons were commercially useless, as they had unpleasant flavors and poor shipping qualities. Crossing the resistant melons with the leading commercial

varieties of cantaloupes or muskmelons, followed by back crossing and repeated selecting, is beginning to give strains of melons which combine the commercial and eating qualities of the American varieties with the resistance of the plants from India. The long growing season in Imperial Valley makes it possible to grow two or three generations each season. In crosses there is a Mendelian segregation of the mildew-resistance character which is at least partially dominant in heterozygous plants. At best 2 or 3 years more will be required to purify resistant varieties of good commercial types.

Three new wilt-resistant varieties of watermelons. DUKE V. LAYTON and J. J. WILSON.

Three wilt-resistant varities, Pride of Muscatine (K–S4), Iowa King (Q23), and Iowa Belle (Q21), grown on 30 acres of heavily *Fusarium niveum* infested soil, produced 27,000 pounds per acre of marketable melons in 1930. Twenty-three acres of Pride of Muscatine yielded 3,111 pounds of seed and showed a wilt resistance, at blossoming time, of 70 per cent as compared with 20 per cent in the commercial checks of the var. Kleckley Sweet. Iowa King in a six-acre field yielded 804 pounds of seed and showed a wilt resistance of 60 versus 5 per cent in the commercial check. Iowa Belle, grown on one acre, produced 175 pounds of seed and gave a wilt-resistance response of 80 versus 23 per cent in the commercial check. Four thousand pounds of seed of the above-named wilt-resistant varieties is subject to distribution.

Iowa Belle showed less anthracnose (*Colletotrichum lagenarium*) injury in greenhouse trials and in the field than either Pride of Muscatine or Iowa King when exposed to infection.

Anasa wilt of cucurbits. L. RAY ROBINSON and B. L. RICHARDS.

Squash and pumpkin growing has been completely abandoned in many parts of Utah owing to a peculiar wilt, resembling in many respects the cucurbit wilt induced by *Bacillus tracheiphilus*. Recent experiments at the Utah station indicate that the wilt is not parasitic in nature but that the squash bug, *Anasa tritis*, is alone responsible for the disease. Wilting results in from 24 hours to 16 days, dependent upon the age of the plant, the progress of the season, and the number of insects feeding. When wilting is not too complete plants uniformly recover upon the removal of insects, and subsequent terminal and axillary growth is always free from disease symptoms.

Wilting results only above the point of insect contact whether the petiole or the entire stem is exposed to feeding. The rate of wilting and the few insects required to produce complete wilting suggest the possibility that a toxic substance injected by the insect during the feeding process is involved. All varieties of squash and pumpkins, together with the watermelon, cantaloupe, and cucumber, appear equally susceptible.

The writers suggest the name Anasa wilt of cucurbits.

Commelina nudiflora, a monocotyledonous host of celery mosaic. S. P. DOOLITTLE.

A mosaic disease, recently the cause of serious losses in Florida, shows characteristics differing from those previously reported on celery, although it is not certain that a different virus is concerned. The unusual feature of the disease is its frequent occurrence on a monocotyledonous host, *Commelina nudiflora*, from which it is transmitted both to celery and cucumber by *Aphis gossypii*, the host in question being a major source of primary infection to celery in the field.

The disease is transmissible by artificial inoculation and by *A. gossypii*, which is an important agent in its dissemination. It is transmissible to cucumber, tomato, tobacco, pepper and ground cherry, the symptoms resembling those of cucumber mosaic, with which the celery virus is probably identical.

The early symptoms are a pronounced leaf mottling and a downward, spreading curvature of the leaf stalks, some of which develop extensive brown, sunken streaks which are especially characteristic of the disease. The later growth of such plants shows little evidence of the disease other than a mild mottling of the leaves and a slight dwarfing. The browning of the leaf stalks, however, may reappear at intervals and often renders the plant unmarketable.

Two Septorias as a cause of late blight on celery. L. C. COCHRAN.

Two distinct species of Septoria have been shown to be responsible for distinct types of the late blight of celery. One causes a large spot, definite in outline, and, under conditions of proper humidity, small scattered pycndia develop in the central portion of the lesion. This type has been identified with the specimens of Briosi and Cavara and with the specimens of Chester (Annual Rept. Delaware Agr. Exp. Sta. 4: 63–65. 1891), which he named *S. apii* (Br. et Cav.) Chester. The other species produces a small spot, indefinite in outline, which is surrounded and densely crowded with black pycnidia. This type has been identified with Dorogin's species which he found in Russia and named *S. apii graveolentis* (Materialye po Mikologia i Fitopatologii Rossii 1: 57–76. 1915.)

Both of these forms have been found throughout the United States, but the type producing the smaller spot (*S. apii graveolentis*) is more common and more destructive. From experiments which are still in progress, both Septorias have been found to be serious parasites of celery leaves but only the small-spot type has been found to infect the leaf stalks.

Comparative histology of three bacterial blights of beans in the seedling stage. W. J. ZAUMEYER.

The relation of *Bacterium phaseoli* and *Bact. medicaginis* var. *phaseolicola* to the tissues of seedling beans is very similar and the organisms are difficult to distinguish except by cultural means. Being Gram-positive and invading the xylem cells to a greater extent than those of the parenchyma, *Bact. flaccumfaciens* is differentiated from the other two, which are Gram-negative and invade the parenchymatous tissues more than the xylem cells.

Bacterium phaseoli and *Bact. medicaginis* var. *phaseolicola* often produce large bacterial cavities in the parenchymatous stem tissues in close proximity to the cotyledonary node, whence they may migrate to the surrounding tissue by way of the intercellular spaces. They may also enter the xylem cells, traveling mostly in an upward direction and may pass through the entire plant, often causing death of the growing tip. Passage of the organisms from cell to cell, either by dissolution of the wall or internal pressure in the vascular tissue, is common.

Similar to the other two organisms, *Bact. flaccumfaciens* may break out from the vascular tissue, causing lysigenous cavities contiguous to the vascular strands from which they came, but the organism does not readily migrate into the surrounding parenchymatous tissues.

A wilt of beans caused by Pythium. L. L. HARTER and W. J. ZAUMEYER.

A wilt of beans caused by *Pythium butleri* was observed and the organism isolated coincidentally at Arlington, Virginia, and Greeley, Colorado, on the Late Stringless Green Refugee variety during the summer of 1930. The disease was first observed about the middle of July on plants from 8 to 16 inches in height. Species of Pythium have been isolated from beans before, but the disease has never been observed under abnormally high temperatures, such as prevailed during the past summer.

The earliest symptoms were observed at the soil level. The decay did not extend much below this point but rather extended upward into the lower branches of the plant. The cortex readily separated from the vascular tissues. The rot was particularly noted at the pulvini of the petioles and leaflets.

A slight flagging of the leaves was first noted in the day time, the plants partially regaining their turgidity at night. A day or two later a distinct wilting occurred, the plants dying soon thereafter.

Refugee beans were successfully infected in the greenhouse and the organism reisolated. The checks remained healthy. Wilting of inoculated plants did not take place at ordinary greenhouse temperatures, but, when they were placed in an infection chamber at a temperature of about 30°C., it occurred in 3 days.

Nature of powdery-mildew injury to snap beans in Virginia in 1930. H. T. COOK.

Powdery mildew caused a loss of over 50 per cent in the fall crop of snap beans in Virginia in 1930. Although a fair yield was obtained, considering the dry season, the beans were unmarketable after the first picking due to a russeting and spotting of the pods by the powdery-mildew fungus. Observations indicate that the disease was less severe on the Refugee than on the Bountiful and Wax varieties.

Correlative studies on the bacteriology of bean mosaic and seed transmission of the virus. RAY NELSON.

Isolations from cage- and field-grown bean plants in 1930 gave the following results: from apparently healthy plants—29 negative cultures from cage-grown Great Northern; 39 negative cultures from cage-grown Robust; 28 negative cultures from field-grown Refugee; 45 negative cultures from field-grown Refugee plants that developed secondary symptoms of mosaic. From field-grown mosaic Refugee plants diseased from seed 56 isolations yielded 27 cultures of micrococci distributed irregularly to the young seed. From rugose mosaic Refugee plants 56 isolations gave 18 cultures of micrococci, 13 cultures of Rickettsia, 21 negative cultures and 2 gross contaminations.

In a study of the progeny from 50 mosaic plants diseased from seed the distribution of mosaic in the seedlings seems to correspond with the irregular distribution of the micrococci to the various seeds of the pod. These studies suggest that the virus is localized in the plant and that its irregular distribution to the seed is due to this localization and to a vascular portal of entry to the ovules. A study of the vascular anatomy of the pod affords supporting evidence for this hypothesis.

The use of fertilizers in reducing losses from pea-root rot caused by Aphanomyces euteiches. C. M. HAENSELER.

The incidence of the pea root rot caused by *Aphanomyces euteiches* was delayed and the injurious effects on the host greatly reduced by the proper use of fertilizers.

Plots receiving 2,000 pounds per acre of a complete fertilizer gave 72.7 per cent increase in plant height and 111.5 per cent increase in yield. An application of 1,000 pounds per acre gave 41.8 per cent increase in size and 89.3 per cent increase in yield. The apparent severity of root rot on these plots by July 1 was rated zero for the 2,000-pound application, medium for the 1,000-pound plots, and very severe on the unfertilized plots. In another test, yield increases of 94 to 206 per cent on Aphanomyces-infested soils resulted from different fertilizer treatments. It was evident in all these tests that much of the increase in yield was due to retardation in development of the disease.

Greenhouse tests have shown that all the principal fertilizer salts tend to retard the development of the disease or decrease the percentage of infection. Nitrate of soda,

sulphate of ammonia, and muriate of potash were more effective in this respect than superphosphate.

Bottom rot of cabbage caused by Corticium vagum. G. F. WEBER.

A destructive and apparently undescribed disease of cabbage was observed in several fields in the vicinity of Gainesville, Florida, during the winter of 1930. The disease was most prevalent in low fields and in wet places in other fields. On individual plants the first symptoms are a browning and dying of the leaves at the basal portion of the head. If the plant is attacked early, the fungus grows rapidly into the head. If the head is older and compact, the fungus grows over the outer leaves, killing them. These leaves are detached and often shed or may remain, giving the head a brown and bald appearance.

The disease is apparently a further development of wire-stem, in which the fungus grows up the stem under humid conditions and attacks the more succulent tissue of the leaf blade. The fungus was observed in the fruiting stage on the host and it compared favorably with *Corticium vagum.* Inoculations made from pure cultures resulted in the reproduction of the disease on potted plants in the greenhouse.

Endohydrosis of forcing cucumbers and its control. RAY NELSON.

A disease of forcing cucumbers characterized by increased hydrostatic pressure, severe water-logging of the leaf tissues, bronzing of the leaf veins, and a general chlorotic and unthrifty condition of the plant has been prevalent in a 3-acre planting at Grand Rapids for the last 3 years. In 1929 the disease was responsible for a crop failure. The leaf spotting usually appears after the first pruning and is most severe during cloudy weather when transpiration is checked. The spotting of the leaves is due to the forcing of water into the intercellular spaces. Shortly after removal from the plant the leaf regains its normal appearance.

This disease apparently is associated with the use of excessive amounts of hen manure as a source of organic fertilizer. Heavy applications of this material are applied yearly to the soil which is then steam-sterilized for four hours. After the first pruning the leaf spotting increases up to the time the fruits begin to set, when this symptom becomes less pronounced. The plants soon become chlorotic and unproductive. Supplementary applications of mineral fertilizer, lacking nitrogen but high in phosphates and potash, have given satisfactory control.

Undescribed symptoms of mosaic in Porto Rico tobacco. MELVILLE T. COOK.

Small black spots resembling those caused by fungi have been observed on the old leaves of tobacco since 1923. No cause was discovered until the winter of 1929–30 when it was found that they occurred on the old leaves of mosaic plants and that they were formed in a very few hours and during the night. Mayer, in 1886, described spots of this kind, but of course it is impossible to say that they are the same. In 1890 Iwanowski and Poloflzoff declared that Mayer had confused two diseases and described these spots under the name of "pokenkrankheit."

These spots are as abundant in the green as in the chlorotic areas but the pattern usually disappears before they are formed. Cytological studies indicate a rapid disintegration of one or more palisade cells which spreads to the mesophyll. The final stage is the collapse of the epidermal cells.

Four apparently undescribed mosaics which go to tobacco. H. H. MCKINNEY.

1. A concentrated yellow mosaic is mild to medium-intense and produces a lacy type of mottling on tobacco. It does not go to tomato and tomato is not a carrier. It is medium-intense on *Nicotiana glauca.*

2. Another concentrated yellow mosaic is very intense and on tobacco and tomato it is indistinguishable from the yellow type previously described. On *N. glauca* the new type is very severe, whereas the previously described one is very mild or does not appear, and its virus is not completely systemic.

3. A green mosaic with a trace of a yellow type is practically indistinguishable from the common mosaic on tobacco. However, on *N. glauca* it is consistently more intense than the latter. All the above viruses are highly potent over long periods and have high thermal-destruction points.

4. Another green mosaic which is free of yellow is mild on tobacco. Mottling is most pronounced on the upper 10 leaves, fading later, causing characteristic premature yellowing on the older leaves. The mottling is unusually pronounced on tomato. It does not go to *N. glauca.* The potency of the virus is low and of short duration, being inactivated at comparatively low temperatures.

Epiphytology of tobacco mosaic in North Carolina. FREDERICK A. WOLF.

A study has been made during the past three seasons to determine by epiphytotic methods: (1) How true tobacco mosaic survives the winter in North Carolina; (2) by what agencies it is introduced into seed beds and into the fields; (3) by what agencies it is disseminated in the fields; (4) what factors govern its rate of spread and its severity; and (5) the applicability of methods of prevention and control. Observations were made on 465 seed beds, 85 of which were mosaic. Two hundred and twenty-nine fields were examined at intervals of approximately two weeks. At the beginning of harvest the average percentage of infection in these 229 fields was 22.7, approximately eight times as great as at the time when the plants had become established following transplanting. By the end of the harvest, the percentage of infection was approximately 95. The primary agent of introduction of the mosaic into seed beds and of dissemination in the fields is man, himself. Mosaic survives the winter, in fields, in the stubbles.

Further studies on virus purification. H. H. MCKINNEY.

Green mosaics from twenty-four sources have been studied on tobacco and related species. All but two of these contained traces of yellow mosaic when collected. In one mixed case the green type has been freed of the yellow type by successive dilutions and inoculations.

These yellow-free green mosaics are distinct from the common type occurring on tobacco and no difficulties have been encountered in keeping them free from yellow mosaic.

Virus of the common mosaic of tobacco has been subjected to dilution tests, chemical and physical treatments, and to tests on various hosts to eliminate the trace of yellow mosaic. All methods have failed and it is considered that we are dealing with a "virus complex."

Leaves from diseased plants grown near 27° C. with continuous light from Mazda lamps in soil of not too high nitrogen content yield virus extracts low in solids and soluble pigments and high in virus potency. The extraneous solids and soluble materials have been removed from these by centrifugation, combined with temperature coagulation, with less difficulty than is usual with extracts from plants grown under ordinary conditions.

Local lesions of mosaic in Nicotiana tabacum. FRANCIS O. HOLMES.

It has been commonly believed that leaves of *Nicotiana tabacum* never show local symptoms when inoculated with the virus of typical tobacco mosaic, and that symptoms appear only on leaves developing after inoculation. During the past three summers, however, yellowish local lesions have been observed and studied in green leaves of Turkish tobacco inoculated with tobacco-mosaic virus. These yellowish lesions proved to be sources of virus in high concentrations before near-by green areas furnished measurable amounts. The distribution of starch was abnormal in the affected areas. Leaves stained in iodine showed conspicuous patterns, which indicated the numbers and positions of points of entrance and multiplication of the virus.

Plastid pigment and chlorophyllase contents of tobacco plants as influenced by three types of mosaic. P. D. PETERSON.

The chlorophyll, carotin, and xanthophyll contents are lowered in tobacco leaves with mild dark green, light green, and intense yellow mosaics. The greatest reduction of all three pigments resulted from the yellow type and the least from the mild dark green type.

The chlorophyllase content of leaf tissues was greatly increased by the intense yellow mosaic in comparison with normal leaves. On the contrary, the chlorophyllase content of leaf tissue yellowed from age or nutritional disturbances was less than that of normal leaf tissue.

The chlorophyll content and chlorophyllase activity of normal leaf tissue seemed to be directly correlated, *i.e.*, the greater the chlorophyll content of the tissues, the greater the chlorophyllase activity. The reverse was true for mosaic-diseased leaf tissue; the light green or yellowed tissues, though lower in chlorophyll content, were higher in chlorophyllase than were the darker green leaf tissues.

There was a progressive decrease in both the chlorophyll content and the chlorophyllase activity of leaves from the top to the base of normal plants. Pith and root tissues were devoid of chlorophyll and evidenced little or no chlorophyllase activity.

Resistance of monocotyledonous plants to Phymatotrichum root rot. J. J. TAUBENHAUS and WALTER N. EZEKIEL.

Root rot caused by *Phymatotrichum omnivorum* attacks more than 500 cultivated and noncultivated species of plants. Monocotyledons have generally been considered resistant, although Phymatotrichum strands were sometimes observed on their roots when near other infected plants. King and Loomis, however, consider presence of strands as evidence that date palms, Johnson grass, Bermuda grass, and sorghum are hosts of root rot. Corn and cotton plants, in alternate hills, were inoculated with fresh cotton-root inoculum. Only the cotton plants succumbed to root rot. Depressed, deep brown lesions were found on roots of check and inoculated corn plants. Isolations from diseased cotton roots yielded typical Phymatotrichum growth. Isolations from the corn-root lesions and similar lesions on roots of sorghum, Johnson grass, corn, and other gramineous crops from root-rot-free or infested areas failed to yield any Phymatotrichum. Lesions of this sort are common in Texas on roots of gramineous plants. Other monocotyledons including canna, caladium, date palm, iris, tuberose, gladiolus, tiger lily, and nutgrass have been grown beside cotton plants and inoculated with root rot. Infection occurred only on the cotton. These experimental evidences suggest that monocotyledonous plants are not hosts to Phymatotrichum root rot.

Nutritional studies on Phymatotrichum omnivorum. WALTER N. EZEKIEL, J. J. TAUBENHAUS, and J. F. FUDGE.

Phymatotrichum omnivorum grows readily in synthetic media, even the sclerotial stage developing in cultures in which the source of nitrogen was ammonium nitrate and that of carbon dextrose. Heaviest growth was secured with a relatively large supply of dextrose and a lesser amount of some source of nitrogen (growth with ammonium nitrate was more than with peptone > urea > glycine > asparagin > potassium nitrate >leucine>ammonium sulphate). Phosphate was essential, also potassium or magnesium or possibly both. Iron, chlorine, and sulphate were omitted without significant change. Growth curves at 28–29° C. reached a peak in 5 weeks with a substratum high in dextrose and in 3 weeks with one of low dextrose content. The media became increasingly acid as colonies increased in weight but tended toward alkalinity, with subsequent degeneration of the mycelium.

In a single series, growth was approximately the same in a complete synthetic medium as with addition of autoclaved extracts from roots of corn (resistant to root rot) or of cotton (susceptible to root rot). Growth was slight but greater with cotton than with corn extracts diluted in water. In undiluted, ultrafiltered cotton-root extract growth appeared more successful than in a similar corn extract.

The resistance of Malvaviscus conzattii (arboreus) to Phymatotrichum root rot. W. J. BACH and J. J. TAUBENHAUS.

Plants of the Malvaceae family, such as cotton, okra, and the common Hibiscus (*Hibiscus rosae-sinensis* L.) are considered very susceptible to root rot caused by *Phymatotrichum omnivorum.* On the other hand, *Malvaviscus conzattii (arboreus)*, commonly known as the Turk's-cap Hibiscus, one of the Malvaceae, appears to be an exception. This perennial, woody shrub is grown extensively in the lower Rio Grande Valley of Texas as an ornamental. It has been under observation for the past eight years, and, although continually growing adjacent to or mixed with other susceptible hosts, was not found dying from Phymatotrichum root rot.

Repeated artificial inoculations of *Malvaviscus conzattii (arboreus)* with pure cultures or with the method reported elsewhere, failed to kill a single well-established plant. Work is now in progress to determine whether resistance is due to possible morphological differences or to some physiological nature inherent in the host.

Development of root rot in cotton planted at different dates. B. F. DANA and H. E. REA.

At Texas Substation No. 5, Temple, Texas, during the 1928 season seven different cotton varieties, ranging from early to late, were each planted at seven dates: March 15, April 1 and 15, May 1 and 15, and June 1 and 15. Root rot appeared at about the same time and increased at nearly the same rate in cotton of the first four planting dates with no differences attributable to varieties. Root rot was delayed in appearance in the June plantings. The total amount of disease at the end of the season was appreciably lessened only in the June 15 plantings. These results indicate that factors other than earliness in the crop govern the time of appearance and rate of development of root rot in cotton. The experiment was repeated in 1929 and 1930 with three varieties planted at three dates. While the disease was less in late-season plantings, yields were unsatisfactory due to drought and insects. It appears that factors favorable for the cotton crop are also favorable for the disease, making it difficult to manipulate the planting date in the control of the root-rot disease.

Cultural and inoculation experiments with Taphrina Potentillae. ELLA M. MARTIN.

During the summer of 1928, a number of cultures of *Taphrina Potentillae* were isolated from plants of *Potentilla canadensis* growing in the vicinity of Ithaca, New York. This organism causes leaf spot of *P. canadensis*. Cultures of *T. Potentillae* on potato-dextrose agar are pale pink, glistening and opaque, and in appearance are very similar to those of *T. Johansonii, T. mirabilis, T. coerulescens,* and *T. deformans.* Cultures of *T. Potentillae* were brought to Greensboro, North Carolina, where they were used in inoculating plants of *P. canadensis.* Inoculation of 38 plants out of doors in March, 1929, and of 16 plants out of doors in March, 1930, produced respectively 50 per cent and 75 per cent of infection. A number of other plants of *P. canadensis* were inoculated with the same cultures in October and November, 1928 and 1929, but no infection resulted, though some of these plants were grown all winter out of doors under cheesecloth and others were grown in the laboratory.

Cytological studies of *Taphrina Potentillae* showed that the development and budding of the ascospores are similar to those reported for *T. coryli* and other species of Taphrina. Ascospores of *Taphrina Potentillae* were found to conjugate in drops of potato-dextrose agar, Sabouraud's agar, decoction of Potentilla leaves, and dextrose agar made with Potentilla leaves.

Progress of Fusarium wilt inside the rhizomes of banana plants. F. L. WELLMAN.

True stems of Gros Michel bananas are bulbous rhizomes with short, horizontally borne, underground stolons. Branches have terminal buds which produce the aerial pseudostem, leaves, and stalk of fruit. Clumps or "mats" of connected plants come from a seed bit cut from a rhizome. *Fusarium cubense* is a vascular parasite producing distinct, readily recognizable, discolored water-conducting tissues. A banana mat becomes infected through uninjured roots or wounds in rhizomes and the disease spreads from plant to plant through underground connections. In dissected rhizomes it is possible to trace the course of the disease many months after infection. It spreads most rapidly inside the rhizome stele, occurring typically at first on one side next to the endodermis. It progresses most easily towards the growing point of the rhizome and from plant to plant in the mat through six generations before symptoms appear in the plant above ground. Buds on the rhizome side affected by the disease are stunted but the undifferentiated tissues are not involved except in very extreme cases. Individuals were found in which Fusarium was recovered in pure culture from roots, rhizome, and leaf sheaths, but not in the fruit stalk attached.

Corm treatments for gladiolus and calla lily. PAUL E. TILFORD.

Various formaldehyde and mercury treatments have been tried on gladiolus corms in an attempt to find a simple and effective measure for controlling scab (*Bacterium marginatum*). The standard treatment, soaking for 2 hours in $HgCl_2$, 1–1000, is difficult when large quantities of many varieties are treated.

Formaldehyde dusts and hot formaldehyde treatments proved ineffective and retarded flower and corm production. Acidulated $HgCl_2$, 1–500, treatments for 5, 10, and 15 minutes were ineffective. Calomel suspension, 1 pound to 2¼ gallons of water proved very effective. Semesan solution, 1 per cent, for 7 hours, controlled scab and stimulated corm production but caused plants to flower later. Organic mercury compounds containing ethyl mercury chloride proved very injurious, a high percentage of the corms were killed, flowering delayed several weeks, and scab was not satisfactorily controlled. Bayer-Semesan compound 694 appears to have possibilities as a short, effective treatment.

The Phytophthora root rot of the white calla lily has been very destructive in Ohio for the last 2 years. Extensive corm-treatment experiments are under way and preliminary results are promising.

Powdery mildew of red clover. CECIL YARWOOD.

The powdery mildew of red clover, *Erysiphe polygoni,* is readily carried in culture on excised red-clover leaflets removed in the late afternoon and floated on a 6 per cent sucrose solution in Syracuse watch glasses. Growth of the parasite is favored by an abundant carbohydrate content of the leaflet. The incubation period is about 6 days. In field plots of equally spaced clover plants of all degrees of susceptibility, a correlation was established between mildew severity and yield. A wide range of resistance and susceptibility was found in seed from the same parent. Two physiologic forms of red-clover mildew have been separated which differ very markedly in their reactions on certain individual plants. As high as 97 per cent germination of spores has been observed. The spores are relatively resistant to the action of certain chemicals but sensitive to sulphur. Germination may occur in a relatively dry atmosphere, although some of the spores and germ tubes may collapse after germination. The germ tubes are negatively phototropic. Spores showing little or no germination can sometimes be stimulated with sucrose solutions to relatively high germination. On the leaflets of a resistant host, reduced germination often occurs.

(Cooperative investigations between the Office of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Botany Department, Purdue University Agricultural Experiment Station.)

The comparative value of checking and drilling in the control of Cercospora leaf spot and yield of sugar beets. I. E. MELHUS and EDGAR F. VESTAL.

Experiments conducted in the field have shown that Cercospora leaf-spot infection is markedly influenced by humidity. Sugar beets are normally thinned to 12 inches in the row, but by increasing this spacing to 20 inches in checked beets the humidity was reduced from 3 to 10 per cent, soil moisture increased about 1 per cent, evaporation increased 3.6 cc. per day, and the wind velocity increased 10 feet per minute over the normally spaced beets. These factors all tend to decrease leaf spot and to increase yield, as is evidenced by the fact that normally thinned beets contained six times as many naturally occurring infection centers and 1,960 pounds of beets per acre less than the checked beets. Analyses of sugar beets from both types of thinning showed a purity of 82.5 and a sugar percentage of 14.56 for the checked, compared to 80.5 and 15.61 for the drilled. However, the increased tonnage of the checked beets more than offset the increased sugar percentage of the drilled.

Checked beets, thinned to one double every other place, and every third place, yielded 33,184 and 33,679 pounds per acre, respectively, as compared to 32,788 and 29,745 pounds per acre, respectively, for checked beets thinned to one to a place and normally drilled beets. Doubles did not reduce the yield.

Alfalfa mosaic.—J. L. WEIMER.

The occurrence of a mosaic of alfalfa has been reported on different occasions to the Plant Disease Survey, U. S. Department of Agriculture. It is believed that these reports have been the records of field observations only. So far as the writer is aware, the existence of a transmissible mosaic of alfalfa heretofore has never been proved experimentally. A mosaic disease of this plant occurs commonly in California and has

been shown to be readily transmissible by aphids (*Illinoia pisi*). Thus far efforts to transmit it mechanically have failed. Evidently the same disease has been found in Wisconsin recently by F. R. Jones who sent affected plants to the writer for comparison with California material. The disease is most prevalent during the cooler parts of the year and is especially abundant in the spring before the first cutting. The losses caused by this disease are very small. So far as observed, affected plants are never killed, the damage being limited largely to a slight amount of dwarfing in the most seriously affected plants. The host range of the virus causing this disease is yet unknown. (Cooperative investigations between the Office of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the California Agricultural Experiment Station.)

Varietal susceptibility, distribution, and control of yellow dwarf of onions. W. J. HENDERSON.

Yellow dwarf of onions is a transmissible virus disease overwintering in the sets and mother bulbs. Field tests in 1929 and 1930 on 35 onion varieties show that the Sweet Spanish varieties possess a high degree of tolerance but are not suited to Iowa conditions. This disease apparently is not localized in Iowa. Inoculations from specimens collected by N. J. Giddings show that it occurs in West Virginia. Giddings has supplied photographs taken in 1916 of what appears to be yellow dwarf. In the autumn of 1929, L. D. Leach sent specimens from California which proved to contain the yellow-dwarf virus.

Indexing onion sets and isolating the set plots have reduced the amount of yellow dwarf in the Pleasant Valley district from 45 per cent in 1928 and 20 per cent in 1929 to 1 per cent in 1930. It is believed that this disease is entirely amenable to control by the methods utilized.

The greenhouse method of indexing sets is being replaced by a water-culture method, in which the sets and mother bulbs are grown on a coarse wire screen resting on the surface of a dilute nutrient solution. This method indicates the amount of infection as well as the greenhouse method and can be carried out under ordinary home conditions.

Virulence of attenuated curly-top virus restored by Stellaria media. C. F. LACKEY.

The virulent form of curly-top virus, attenuated by passage through nettle-leafed goosefoot, *Chenopodium murale,* is restored to approximately its original virulence by passage through chickweed, *Stellaria media.* The following results are representative of those which demonstrate this fact:

Kind of virus	Number of beets inoculated	Per cent diseased	Average incubation period in days	Severity of symptoms
Original (virulent)	65	64	9.5	Severe
Attenuated	83	25	13.1	Very mild
Restored	110	74	9.9	Severe

The symptoms produced by the restored virus were indistinguishable from those produced by the original, virulent virus. These consisted of severe dwarfing and distortion of the leaves. The attenuated virus by contrast produced very slight dwarfing and distortion.

Chickweed is probably not an important factor in restoring the virulence of the virus in nature because it grows in moist and shady places which are unfavorable for the beet leaf hopper, *Eutettix tenellus*, the vector of the virus.

Filaree, *Erodium cicutarium*, however, is a very important host for the leaf hopper in California during winter and early spring. It also plays an important rôle in the overwintering of the virus. Investigations are now in progress to determine its effect on the attenuated virus.

New virus diseases in Porto Rico. MELVILLE T. COOK.

Brief descriptions of six unreported virus diseases follow. A mosaic of *Crotolaria striata* dwarfs the plant and reduces seed production, but it is not carried in the seed. A rare mosaic of *Commelina longicaulis* appears only when the plant is making a vigorous growth and does not injure the plant. A bunchy top of *Papaya carica* appears to be due to a virus. It is very destructive but easily eradicated. A variegation of *Abutilon hirtum* is quite common. It dwarfs the plant to some extent and reduces seed production, but it is not carried in the seed. A variegation of several species of Sida may be due to the same virus. A mottling of the leaves of several species of mulberry appears to be due to a virus. It can be transmitted by scions.

The effect of ultra-violet radiation upon representative species of Fusarium. ALICE A. BAILEY.

Ultra-violet radiation has been found effective in increasing the amount of sporulation and the percentage of macrospores produced in the various species of Fusarium causing bulb rot of onions. Strains which usually produce mostly microspores in artificial culture produce abundant normal macrospores after radiation. In the case of a strain that had never produced any spores in culture during the 4 years succeeding isolation from a decayed bulb, macrospores were produced in abundance after radiation.

Because of its value in the work on taxonomy of onion Fusaria, radiation has been tried on some 25 species of Fusarium, representing all but one of the sections, in the hope that macrospores could be produced in such species as ordinarily produce only microspores in pure culture. In most instances radiation increased the total number of spores and the percentage of macrospores, where macrospores are characteristic of the species. In some few cases radiation reduced the number of spores. Some filters are more effective than others in screening out rays of detrimental wave length and permitting advantageous rays to pass through.

Plant extracts and fungi. II. Bean extracts in relation to Colletotrichum lindemuthianum. E. S. REYNOLDS and B. S. MILLER.

Extracts were made from bean plants which had been dried artificially at about 80° C., and then ground to a fine powder. An amount of water equivalent to the water content of the growing plants was used and mineral salts and glucose were added. Liquid cultures made with these undiluted extracts from certain varieties of young bean plants have been found to prevent growth of *Colletotrichum lindemuthianum*. Agar plates made with similar extracts supported a slowly developing growth of the fungus. Check cultures, having minerals and glucose in the same proportions as used in the bean-extract cultures, produced a good growth of the fungus. Preliminary studies indicate varietal differences in quantity of toxic action.

A destructive fungous disease of the corn borer. C. L. LEFEBVRE.

During the past year an epidemic of *Beauveria Bassiana* on larvae of the corn borer was observed in the European Corn Borer Laboratories at Arlington, Massachusetts, the

mortality among the larvae arising as high as 90 per cent in lots imported from Manchuria.

The occurrence of *B. Bassiana,* to the writer's knowledge, has never been reported on the corn borer in the United States. In laboratory experiments, 100 per cent larval mortality is obtained within two days when inoculated with conidia of *B. Bassiana,* while in experiments with *B. globulifera,* which is common in the United States, only 4 larvae were killed in seven trials of 10 larvae, each. Preliminary field tests were made, in which spores of *B. Bassiana* were dusted on corn-borer-infested fields. These trials indicated that at least a partial control of the corn borer can be obtained. The disease is first noted by the infected larvae turning pink from which the malady gets its name the *Pink Disease.* The larvae soon become mummified, and after a few days a white mycelial outgrowth is evident, which turns to a creamy, powdery mass, due to abundant spore formation.

B. Bassiana on artificial media is characterized by a flat, mealy, pulverulent growth, forming conidia in abundance within several days. *B. globulifera* on artificial media produces an elevated, cottony, floccose growth, not forming conidia for several weeks. In Van Tieghem cells, spores of *B. globulifera* produce much more extensive germ tubes which branch profusely throughout the droplet as compared to *B. Bassiana.* On artificial media, *B. Bassiana* does not readily lose its virulence.

Sclerospora butleri, a new species from Nyasaland. W. H. WESTON, JR.

The fungus was found on the wild grass, *Eragrostis aspera,* among tobacco plantings at Bulaki, Nyasaland, South East Africa, by E. J. Butler. It is characterized by the fact that the oospores (17 to 25 μ diam.) are smaller than any yet known, while the surface of the dark amber oogonial wall instead of being roughly patchy or ridged, angled and polygonally-faced as in other species, is marked by pallid, rounded protrusions 2 to 3 μ broad and 2 to 5 μ high.

The host shows the well-known shredding of the leaves which are infested by the resting spores only. The conidial phase is being sought to determine whether it produces zoosporangia germinating by zoospores as in typical *Sclerospora graminicola* or conidia germinating by hyphae as in other species.

As yet the fungus is known only from this collection on this grass, but the host has a wide range, occurring throughout Africa even to elevations of 2,000 meters and extending into southern India.

The Dutch elm disease in Ohio. CURTIS MAY, O. N. LIMING, and THELMA ALEXANDER.

Five cases of the Dutch elm disease were found on American elm in Ohio during the past summer. Symptoms were a wilting of leaves and new growth followed sometimes by yellowing and defoliation and at other times by browning of leaves. One or more annual rings showed a spotted brownish discoloration. Verticillium wilt of elm, because of similar field symptoms, can be distinguished from the Dutch elm disease only by cultures.

The fungus, isolated from roots, trunks, and twigs was culturally and morphologically similar to that described for *Graphium ulmi.* Buisman confirmed this identification, and Koch's rules of proof were satisfied.

In culture the colonies enlarged most rapidly at pH 4.8–5.9 and from 19°–27° C. They were yeast-like at pH 7.4–8.4.

Mycelium of the fungus was found abundantly in the tracheae, wood parenchyma, fibers, and wood rays of inoculated trees, but in naturally infected trees it was not common and was found only in the tracheae. It is intra-cellular and apparently passes from cell to cell through the numerous pits.

New methods for determining rate of decay behind cavity fillings in trees. W. HOWARD RANKIN.

Cavity methods should be judged by the subsequent rate of decay because complete eradication of the fungus is probably rarely accomplished and often unwarranted. Wound decays, naturally sealed in by callus growth, are known to be arrested. An effective cavity treatment should accomplish the same result if it seals the opening. Initiating decay in small cavities dug in sound wood by imbedding blocks of wound-decay material has been attempted in an extensive series in oaks and maples. To study the fundamental factors, apparatus has been installed in filled cavities which makes possible the insertion and withdrawal of pure-culture material without disturbing the filling or callus. This apparatus also provides for the analysis of the gases in the wood. The carbon dioxide content of trees and of the wood behind various types of fillings is being studied as the possible factor accounting for the cessation of decay in closed wounds. The X-rays have been used successfully to show accurately slight amounts of decay in living trees. Radiographs provide a means of annually recording the rate of decay behind fillings of different types not only in experimental cavities but also in those of unknown previous history.

Use of oxides of unsaturated hydrocarbons for the eradication of barberries and other pests. R. B. HARVEY.

Practical applications have been made of ethylene oxide and propylene oxide for killing barberries, gooseberries, and other noxious plants. Ethylene oxide diffuses well in soil and into the plant, producing death in one or two weeks. The stem tissues blacken and leaves fall off, indicating death of the plants. A "depth charge" is injected by means of prod rod provided with a measuring chamber. With this "gopher stick" a measured charge is injected into the soil beneath the plant, using the pressure of the ethylene oxide directly from the tank, or a solution of ethylene oxide in water can be injected from the usual knapsack sprayer.

Corynose twig blight of the American bladder nut. W. H. DAVIS.

During 1929–30, a severe twig blight of the American bladder nut, *Staphylea trifolia* L., was observed in Massachusetts. Some of the smaller shrubs were killed while half the current year's growth of others was destroyed.

During April, 1930, monosporous cultures of the associated fungus were made and incubated on steamed oat-meal, steamed yellow corn-meal and potato-dextrose agars. Conidia and mycelium from these cultures were employed in making inoculations during May and July. Infections showed that the fungus was pathogenic and hyphae entered through meristematic tissues of twigs at the tips, nodes, or leaf axils.

The fungus somewhat resembles *Coryneum microstictum.* Conidia were sub-pyriform; 6 x 18 μ (Saccardo; 5–6.5 x 15–17 μ); apex obtuse, 4 loculi, lowest loculus sub-hyaline; those above, honey-color; conidiophores, filiform, hyaline. However, these differences were noted; stroma present, not absent or obsolete; conidiophores, 1.3 x 19, not 1.5 x 20–25 μ. *Staphylea trifolia* is an unreported host. The classification suggested for the fungus is: *Coryneum microstictum* B. and Br. variety, staphyleae.

A survey for stinking smut in wheat. R. J. HASKELL, R. C. ROSE, W. E. BRENTZEL, E. A. WALKER, and WALDO KIDDER.

An examination of 814 wheat fields comprising 66,729 acres in 17 counties of Minnesota, the Dakotas, and Montana, in 1930, has shown that 62 per cent of the spring- and 92 per cent of the winter-wheat growers treated their seed. The average percentage of

smut was: spring wheat treated 2, untreated 4, total 2.8; winter wheat (110 fields in Montana) treated 5.9, untreated 24.8, total 7.4.

Formaldehyde was used on 74 per cent of the spring and 25 per cent of the winter wheat; copper carbonate on 20 per cent of the spring and 55 per cent of the winter.

The percentages of smut in spring wheat following different treatments were: 50 per cent copper carbonate with machine, 0.3; 20 per cent copper carbonate, commercial machine, 0.5; formaldehyde, machine, 0.9; 20 per cent copper carbonate, home made machine, 1.1; Ceresan, machine, 1.3; Ceresan, shoveled, 1.3; formaldehyde, dip, 2.0; formaldehyde, sprinkle, 2.1; 50 per cent copper carbonate, shoveled, 3.2; 20 per cent copper carbonate, shoveled, 4.6. Winter wheat results were comparable. Spring wheat treated annually had 1.4 per cent. smut; not treated annually, 4.7 per cent. The leading spring wheats showed the following percentages of smut in untreated fields, Ceres 9, durum 7, Marquis 4, Ruby 2, Marquillo trace, all hard red spring wheats 2.7, all durums 2.9.

Effect of time and rate of application of seed disinfectants on oats and wheat. BENJAMIN KOEHLER.

Tests were conducted for 2 years with formaldehyde dust (Smuttox in 1929, Corona Oat Dust in 1930) on 2 varieties of oats. Treatments made 3 months, one month, and one week before seeding all caused reductions in yield of grain as compared with treatments made one day before seeding, the depression being in proportion to length of time. Treatments made with Ceresan 3 months or one month before seeding also caused some depressions in yield.

On smutty seed, all these treatments, however, caused increases in yield ranging from 0.7 to 10.7 bushels for formaldehyde dust and 3.9 to 14.9 for Ceresan, according to time of application. On seed nearly free from smut the long-time treatments caused a loss in yield. Three ounces per bushel controlled oat smut better than 2 ounces.

Ilred wheat was treated with copper carbonate, extended copper carbonate, Ceresan, and other materials during the last 2 years. The seed contained some Gibberella infection both years, but no bunt infestation. Significant increases in yield ranging from 2.4 to 11.7 bushels per acre were obtained with the compounds named. Two ounces per bushel of any one of them proved just as effective as 3 ounces.

Wheat diseases in Tennessee. C. D. SHERBAKOFF.

Sulphur dusting for the prevention of a black chaff of wheat. F. J. GREANEY.

In testing the effectiveness of sulphur in controlling leaf rust *Puccinia triticina* Eriks., and some of the minor leaf and stem diseases of wheat, it was found that frequent applications of sulphur dust prevented the development of a bacterial disease of wheat, called black chaff.

At harvest time the percentage of nondusted wheat plants infected with black chaff ranged from 65 to 95, with an average of 75 per cent; whereas the range of the dusted plants was from a trace to 8 per cent, with an average of 4 per cent. In the absence of significant amounts of leaf and stem rust the statistically significant increased yield of 5.9 bushels per acre resulting from dusting was due in a large part to the control of black chaff.

The results of the sulphur-dusting experiment suggest very strongly that the organism causing black chaff spreads from plant to plant in the field. Wind and rain probably are the most important agents of dissemination. This disease has not been positively identified as the black-chaff disease caused by *Bacterium translucens* var. *undulosum*, but in general symptoms they are identical.

Cold injury. J. R. HOLBERT and W. L. BURLISON.

Progeny studies, in the field, of yellow dent corn plants grown from mature seed unexposed and exposed ten hours to three different temperatures, 32, 23, and 14°F., respectively, in field refrigeration chambers in the fall of 1929, indicate that seed value of some strains of corn, with 20 per cent moisture in the grain, was injured by exposure to temperatures of 32° F. and lower. However, there were marked differences between different inbred and crossbred strains. Germination studies and field yields indicate that germination does not always give reliable index of extent of cold injury.

As determined by temperature measurements by thermoelectrical methods, different corresponding tissues of corn plants of different strains differed greatly in rates at which they cooled when exposed to chilling and subfreezing temperatures in refrigeration chambers. Husks of some strains offered more protection to shanks and grain than did husks of other strains.

Application of phosphate to soil prior to planting greatly increased cold resistance of young corn plants of both cold-resistant and cold-susceptible strains.

Three varieties of soybeans, Wilson, Virginia, and Illini, differed greatly in their resistance and susceptibility to cold injury in the late maturation stages. (Cooperative investigations by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Illinois Agricultural Experiment Station, and Funk Bros. Seed Company.)

Relation of seed quality to yielding ability and disease resistance in hybrid strains of dent corn. R. R. ST. JOHN and J. F. TROST.

Comparisons were made of the field performance of crossed seed selected from healthy inbred or crossbred plants with similar seed selected from corresponding diseased plants, all grown under conditions of continuous corn culture, on similar soil, with an abundance of natural inoculum of the various root-, stalk-, and ear-rot fungi. These seed-quality comparisons involved 37 distinct hybrid combinations of dent corn. Weather conditions favored abundant stalk and ear infection by *Diplodia zeae.*

As a grand average for all 37 hybrids, plants from seed from healthy plants yielded 4 per cent more than the plants from seed from diseased plants. In paired comparisons, the seed from healthy plants was superior 84 out of the possible 111 times.

There was no significant difference in the percentage of nubbins, broken stalks, or ear rot caused by *Diplodia zeae.*

The very best crossed seed of hybrid strains that were either too early in maturity or were still immature at the time of killing frosts did not yield so well as ordinary low-quality crossed seed from diseased plants of those hybrid strains that matured by the time of killing frosts. (Cooperative investigations by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Botany Department, Purdue University Agricultural Experiment Station.)

Resistance in sweet corn to Diplodia zeae. GLENN M. SMITH and JOHN F. TROST.

In 1930, field trials of inbred and crossbred strains of sweet corn were conducted on land that had been in continuous corn culture for ascertaining the relative resistance to natural infection by the various root-, stalk-, and ear-rot fungi. A comparison of the amount of Diplodia infection in sweet corn and in dent corn grown in the same field showed 7.5 per cent ear infection for 500 strains of dent corn and 10 per cent ear infection as an average of 225 strains of sweet corn. Apparently, sweet corn is inherently as resistant to ear rotting by *Diplodia zeae* as is dent corn.

In these experiments 65 strains of sweet corn showed less than 5 per cent ear infection by *Diplodia zeae,* while 23 strains showed more than 30 per cent.

Inbred lines, selected solely on the basis of horticultural performance during the first five years of their inbreeding, had a significantly higher percentage of ear infection than those inbred lines selected for disease resistance throughout their inbreeding.

In a number of instances where both parents of single-cross hybrids were highly susceptible to ear infection, the hybrids were nearly free from such infections. (Cooperative investigations by Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Botany Department, Purdue University Agricultural Experiment Station.)

Cornstalk rot and ear rot. A. L. SMITH and J. R. HOLBERT.

Stalk and shank tissues of nearly mature corn plants injured by exposure to low temperatures in field refrigeration chambers have been found to be much more susceptible to infection from artificial inoculation with *Basisporium gallarum* than comparable tissues of corn plants not injured by cold. Likewise, stalk and shank tissues of corn plants grown on old soil were more susceptible than were comparable plants grown on new soil.

Marked differences in relative resistance of stalk and shank tissues to infection from artificial inoculation with *Diplodia zeae* were found in both inbred and crossbred strains of corn.

Stalks of corn plants grown on more productive soils were more resistant to natural infection from *D. zeae* than stalks of comparable plants grown on less productive soil. (Cooperative investigations by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Wisconsin Agricultural Experiment Station, and Funk Bros. Seed Company.)

Nodal infection with the corn-smut organism. I. E. MELHUS and GLEN N. DAVIS.

When 500 sweet-corn plants, variety Golden Bantam, one foot tall, were inoculated with two opposite monosporidial suspensions in carrot decoction, having a surface tension of 47 dynes, 33 per cent of the plants became infected. When the surface tension was changed to 34.4 dynes, by the addition of fish-oil soap, 91 per cent became infected. No smut boils developed on the lower nodes in the former case, while 40 per cent of the infected plants in the latter produced smut boils; and 54.5 per cent of the smut boils produced did not express themselves until after the plants had tasseled.

When three inbred lines, reputed as very resistant, showing an average of only 1.4 per cent natural infection in the field, were inoculated with a low surface-tension spore suspension, boils were produced on 35 per cent of the plants.

In both field and greenhouse experiments, when the leaf sheaths were removed late in the development of the plant, many were found where infected axillary buds existed with little hypertrophied tissue. It is believed that all axillary bud infection resulted from spores dropping into the spiral whorl and manifestations of the infections were only in those buds which became active through any cause.

Basisporium dry rot of dent corn as related to temperature and cob reaction. C. S. REDDY.

From many experiments in which Basisporium-infected dent corn seed was germinated at different temperatures and in which different strains of corn were germinated at the same temperature, it was apparent that severe injury occurred in the form of seed rotting when the temperature relation to the seed prevented early germination. No apparent injury occurred immediately after germination; therefore, there is no seedling-blight stage.

Susceptibility to Basisporium ear rot was directly correlated with low acidity and resistance with high, as determined in distilled water extracts of disease-free cobs.

Cob pH values	Inbreds in class interval	Ears observed	Ears infected
	No.	No.	Per cent
4.4–4.7	5	116	0
4.8	6	121	2.5
4.9–5.0	14	312	7.4
5.1–5.2	16	313	22.7
5.3–5.4	12	258	38.0
5.5–5.6	7	175	41.7
5.7–5.8	8	185	33.5
5.9–6.3	7	173	48.6

These two tests are especially suitable for use by corn breeders, because, in their application, they require no knowledge of plant diseases and may be used every year whether or not there is an epidemic of Basisporium ear rot.

Fifth progress report on studies of fall applications of fungicides in relation to apple-scab control. G. W. KEITT.

Penetration and toxicities of petroleum-oil sprays. P. A. YOUNG.

Apple and potato leaves partly soaked with pure oils (6 per cent or less sulphonatable residues) lived for 30 to 80 days. Pure oils with more than 13 per cent sulphonatable residues killed apple leaves within 7 to 14 days; they were toxic to apple and potato leaves in 4 to 8 per cent oil sprays, being much more injurious than nearly saturated oils. Winter killing was associated with abundant oil in apple twigs. Oils spread between parenchyma cells and in tracheae of apple limbs, leaves, and fruit. One per cent sprays with nearly saturated oils caused no macroscopic symptoms in apple or potato leaves; eight per cent sprays caused some black sports in potato leaves. Oils spread mainly between parenchyma cells in potato and cucumber stems. Oils passed from potato leaves into the tubers. Protoplasm flowed for eight hours in staminal hairs of *Tradescantia fluminensis* immersed in pure, nearly saturated oils. *Helminthosporium sativum, Rhizopus nigricans, Achlya conspicua, Glomerula repens,* and *Penicillium* sp. grew abundantly and anaerobically in pure oils. They grew best in nearly saturated oils. Earthworms (Annelida) and larvae of *Phorbia brassicae* lived for a few hours in pure, nearly saturated oils but not in less saturated oils.

The relation of pentathionic acid and its constituents to the toxicity of sulphur fungicides. O. NEAL LIMING.

Since 1928 a further study has been made of the chemical and physical nature and the toxicity of pentathionic acid and of its constituents, sulphur, sulphur dioxide, and hydrogen sulphide. Although the toxicity of sulphur to fungi has been attributed to a number of factors, only volatilized sulphur, sulphur dioxide, hydrogen sulphide, and pentathionic acid are now usually considered.

The volatile product of sulphur is a vapor. At summer temperatures, over ten per cent of the sulphur on a dusted surface may pass off as a vapor within two weeks.

Sulphur vapor is not toxic to fungus spores, and the condensation products are toxic only after standing several hours. Sulphur dioxide occurs only in traces in ground sulphur and is not toxic in such concentrations. Hydrogen sulphide does not occur in ground sulphur. Traces of this gas are produced from sulphur when in contact with higher plants and fungi, but it is not toxic to fungi in such conentrations. Pentathionic acid is associated with ground sulphur and in concentrations toxic to fungus spores. It is a natural oxidation product of sulphur but its formation is enhanced by mild oxidizing agents and possibly by hydrogen sulphide. It is not sufficiently volatile in dilute solutions to be toxic at a distance. The pentathionate ion is stable in acid and weak alkaline solutions but is toxic only in acid solutions. The toxic action is governed by the condition of the fungus rather than by the condition of the pentathionate ion.

Water-soluble arsenic in spray materials. H. C. YOUNG.

When lime sulphur and lead arsenate are mixed together in the proportion generally used for spraying a black sludge primarily composed of lead sulphide is produced. As lead sulphide is formed a portion of the arsenic becomes free. It was found that hydrogen sulphide normally present in lime sulphur is primarily responsible for the breaking down of lead arsenate and that this reaction is accelerated as the line sulphur is diluted. The arsenical determinations were made on spray materials dried on rubber mats at constant humidity and temperature. An attempt was made to duplicate actual spraying conditions and to obtain results comparable to actual field spraying. The water-soluble arsenic ranged from 6 per cent in the 1–40 lime sulphur dilution to 12 per cent in the 1–80 dilution.

An attempt was next made to add materials that would check the above reaction. It was found that calcium hydrate was the only effective one out of the following list: calcium carbonate, calcium chloride, ferrous sulphate, aluminum sulphate, aluminum hydrate, calcium caseinate, zinc hydrate, barium hydrate and carbonate and magnesium limes.

A mixture of calcium monosulphide and arsenate of lead resulted in a high percentage of free arsenic. Other fungicides mixed with arsenate of lead, including Dry mix, Sulfuron, Mulsoid sulphur, and Mist Brand wettable sulphur were also tested.

Results with new sulphur dusts for apple-scab control. A. L. PIERSTORFF and H. C. YOUNG.

The failure of most sulphur dusts to control apple scab during seasons favorable for the development of the fungus is attributed to their failure to adhere evenly to the foliage and fruit surfaces, and to insufficient toxicity.

In an attempt to overcome these difficulties two new dusts have been developed; namely, 85–15 sulphur—dry lime sulphur and 85–10–5 sulphur—manganar—aluminum-hydrate. These two dusts adhere to the foliage better and are more toxic to the apple-scab fungus than ordinary dusting sulphur. In three years' tests, two of which have been severe scab seasons, these dusts produced in 7 orchards 92.3 per cent clean fruit for the sulphur—dry lime sulphur and 85.7 per cent clean fruit for the sulphur—manganar—aluminum hydrate, while the plots dusted with a commercial-sulphur dust had 71.6 per cent clean fruit. The checks averaged 14.5 per cent clean fruit. The same variety was used for all plots. The dusts were applied throughout the season with the exception of a delayed dormant spray.

Hydrogen sulphide as related to the fungicidal action of sulphur. S. E. A. MCCALLAN and FRANK WILCOXON.

Hydrogen sulphide is exceedingly toxic to fungus spores and is evolved when sulphur is applied to the surface of spores and leaves of higher plants.

The germination of conidia of *Venturia inaequalis*, of uredospores of *Uromyces caryophyllinus* and *Puccinia antirrhini*, and of conidia of *Sclerotinia americana*, *Macrosporium sarcinaeforme*, *Pestalotia stellata*, *Glomerella cingulata*, and *Botrytis cinerea* is completely inhibited at hydrogen sulphide concentrations ranging, respectively, from 0.2 to 40 milligrams per liter of solution. This is also the identical order of sensitivity of these fungi towards sulphur.

The evolution of hydrogen sulphide has been demonstrated from all species tested—19 fungi and 26 higher plants. The amounts produced by known quantities of spores and by strawberry plants have been determined. The production by spores appears dependent on the quantity of spores, increases with temperature to about 30° C., ceasing at 65° C., and occurs over a pH range from 4.0 to 8.0 with no well-defined optimum. Spores of Sclerotinia and Glomerella produce, respectively, 5 and 15 per cent of their weight of hydrogen sulphide in six hours.

Indications point to the reduction of sulphur on or within the spores and its initiation by glutathione. Glutathione has been demonstrated in spores of Sclerotinia.

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STUDIES ON A RUST OF CLINGSTONE PEACHES IN CALIFORNIA

M. C. GOLDSWORTHY AND RALPH E. SMITH[1]

The commercial canning of peaches and the production of fruit for this purpose constitute an industry almost entirely confined to California. Statistics presented by Wellman (38) show that since 1921 California has produced more than 97 per cent of the canned-peach output of the United States. During the past decade there has been a tremendous increase in the planting and total pack of clingstone varieties, accompanied by a considerable decrease in the output of canned freestone peaches. The following figures are taken from the California *Fruit News*, June 15, 1929.

Number of cases of peaches canned in California

	1921	1922	1923	1924	1925	1926	1927	1928
Freestone	1,633,418	1,314,597	872,676	963,621	1,198,314	817,319	320,812	163,830
Clingstone	4,162,849	7,844,912	6,591,335	5,366,598	9,258,587	13,654,758	10,829,681	14,811,606

Peach trees are susceptible to many diseases, among which that known as rust has become of great potential importance to the clingstone-peach orchards of California. During the three years previous to 1928 serious outbreaks of this disease occurred in various parts of the State, especially in that portion lying along the Yuba, Feather, and Bear rivers in Yuba and Sutter counties, which, because of the character of the soil and climate, is particularly adapted to the production of quality fruit and maximum tonnage. The region is devoted to the culture of the clingstone varieties and the crop is handled entirely through the canneries.

The disease under discussion has been known in this region and in other parts of California for many years (Scribner (33), 1887; Pierce (32), 1894) but usually on such a minor scale that its occurrence was thought to be of only passing significance as an indication of climatic conditions un-

[1] Acknowledgment is due to Professor W. P. Duruz (20, 21), whose spraying experiments on the control of peach rust were closely associated with this work.

usually favorable to the fungus but not likely to occur regularly or frequently. During 1925, 1926, and 1927, however, such serious losses resulted from this cause that the growers and canners became deeply concerned at what really seemed to be a new and serious menace to the industry.

This fungus disease, a true rust, has occasioned unusual alarm because it not only caused leaf injury and more or less defoliation, as is usual with rusts, but, particularly in 1926, entailed immense losses of fruit, the peaches being marked with rust lesions and rendered unfit for canning. Many growers thus lost their entire crop of certain varieties. The fact that the new, so-called midsummer clingstone varieties, extensively planted in recent years, have been more susceptible to this disease than the older varieties increased the seriousness of the situation.

Since 1927 almost no loss has been caused by rust in peaches. It therefore is natural to assume that the outbreak described was of a sporadic nature and that the reasonable expectation regarding this disease is one of only occasional and irregular occurrence. Experience with other plant diseases, however, has shown that it is not safe to rely too strongly on this assumption without the reassurance of a thorough knowledge of the nature of the disease and methods for its control. The fundamental relation of rainfall and relative humidity at certain seasons to the development of rust, thus explaining its sporadic occurrence, is brought out in this paper.

DESCRIPTION OF THE DISEASE AND THE CAUSATIVE FUNGUS

Cursory examination shows that this disease is caused by one of the true rust fungi (Uredinales), characterized most commonly by the production of an abundance of urediniospores.

Leaf phase (Fig. 1). This is the most frequent form of the disease and may become at times so severe that complete defoliation occurs. The leaves never become entirely covered with rust spots, but, as a rule, these are either scattered irregularly over the entire surface or confined to portions of the leaf. The areas first attacked become evident as pale, yellowish-green spots clearly defined on both surfaces. These spots are angular in outline and apparently are limited by the larger veins. Later these spots take on a bright yellow color, and the lower surfaces, rarely the upper, become conspicuous by the presence of dusty, dark brown rust sori in the central portion of the chlorotic area. These sori are filled with the cinnamon brown urediniospores.

When lightly affected, the leaves do not prematurely drop but remain on the trees until the normal fall in early winter. When heavily affected, that is, when most of the leaf surface is occupied by diseased areas, abscis-

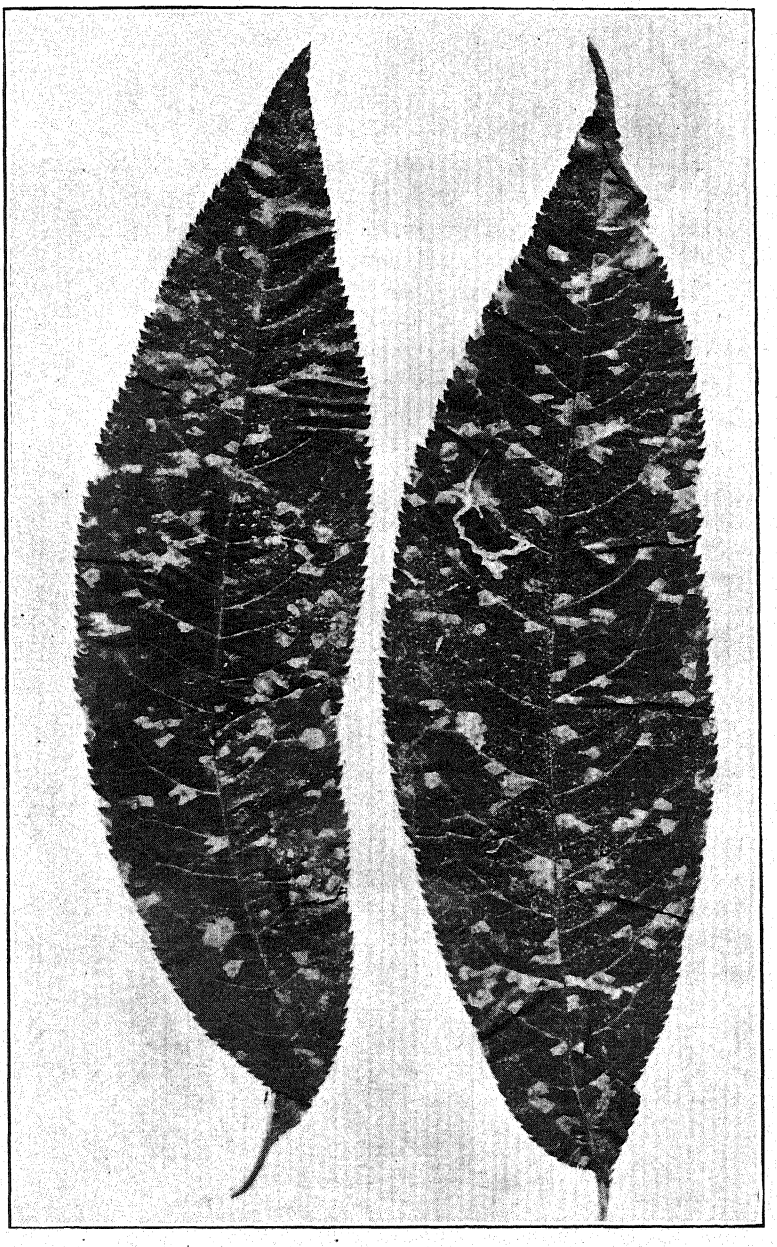


FIG. 1. Peach rust; leaf phase.

sion may occur and a partial to almost complete defoliation result. The effect of the organism upon the host is local and confined to a relatively small area of the mesophyll. The affected area does not collapse for some time and the first indication of a decline is the disappearance of chloroplastids which, no doubt, accounts for the color change in the rust spots on the leaves. After the fungus fruits the affected regions appear, for a time, greater in thickness than the adjacent normal portions of the leaf. This phenomenon is due to the presence of intercellular mycelium and a stromatic layer of pseudoparenchymatous tissue directly beneath the epidermis and not to an increase in the number of cells. Later these central portions

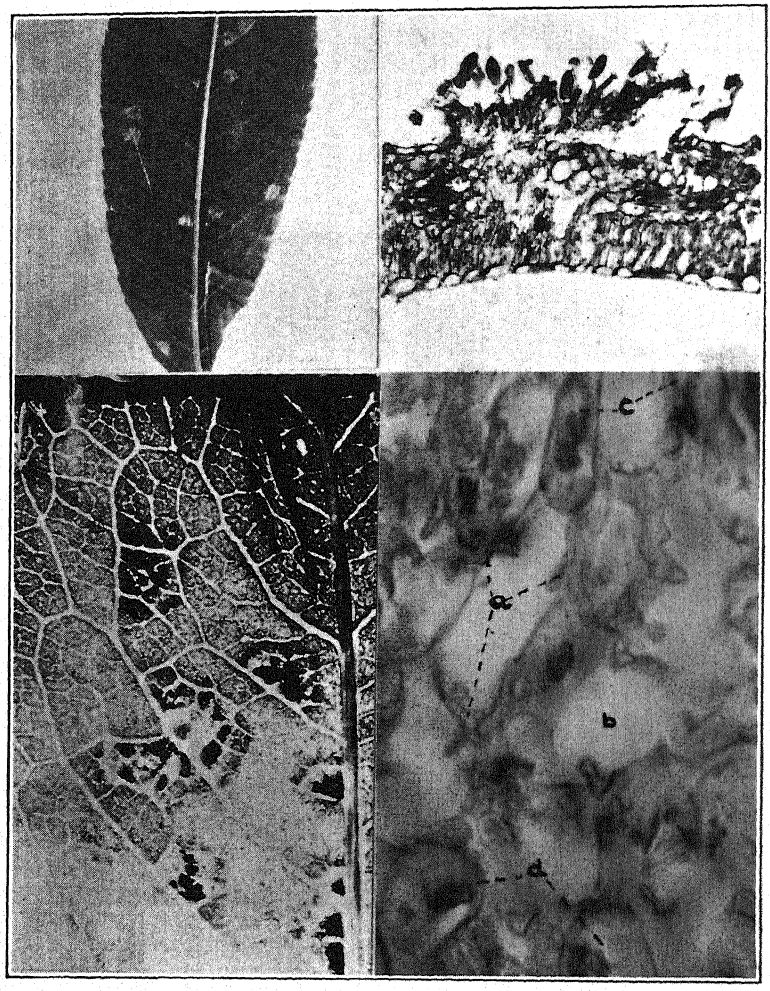


FIG. 2. Peach rust on leaves and sections of leaves showing sorus and affected mesophyll.

of the affected regions shrink, the cells take on a plasmolyzed appearance and during periods of high temperature they dry out, forming in some cases dead areas. The fungus continues, however, to live in the marginal tissues of the affected areas and later, as the cool weather of the fall comes on, new rust sori are formed in this part of the old lesion. The dead areas do not absciss, as in shothole diseases, but remain firmly attached.

Mycelium. The fungus invades the tissue with the formation of intercellular mycelium, the filaments containing many nuclei and with a relatively small diameter. In no case examined was the diameter of the hyphae greater than 8 μ and in most cases it was less and exceedingly irregular. The fungus forms abundant haustoria, which attain various sizes. Many of these are found in the cells and as many as six have been seen in a single large cell, such as is found in the peach hypodermis (Fig. 2, a).

Urediniospores. Spore production is characterized by the formation of a stromatic layer of pseudoparenchymatous tissue which, in the leaves and bark, is formed directly beneath the epidermis and, in the fruit, at various distances beneath the epidermal layer. From this layer mycelial strands are formed, woven into a more or less compact superstructure, at the apex of which each strand forms either a paraphysis or a urediniospore (Fig. 3, A).

The paraphyses are long, thin cells, bulging at their apices into rounded ends with a thickened brown covering. These vary in size, the longest examined measuring 32.6 μ, the shortest 17.6 μ. The number of paraphyses in a sorus appears to depend upon its maturity, for in young sori more of these structures are present than in the older, and, as a matter of fact, in the later cases it is quite common to find no paraphyses.

The urediniospore is borne on a stalk consisting of a basal cell and a short pedicellate cell. The urediniospores are cinnamon brown in color, mostly truncate, often elliptical and rarely isodiametric, made conspicuous by a thick, smooth apical tip of varying dimensions, sometimes covering as much as a third of the longitudinal diameter. They are heavily echinulate except at the apex, usually sharply constricted at the base, pedicel not adhering, heavily filled with granular protoplasm and oil inclusions and with pigment apparently contained in the outer covering. There are two germ pores, usually located beneath the apical cap and opposite each other. Measurements of 1,000 spores from various sources gave an average maximum-minimum of 33.6–12.2 x 14.4–9.0 μ. Typical urediniospores are shown in figure 4, B.

Bark phase. The bark phase of the disease (Fig. 4, A) is a relatively uncommon one in the Sacramento Valley and because of this has evaded regular observation. On many small, current-season twigs, marked as

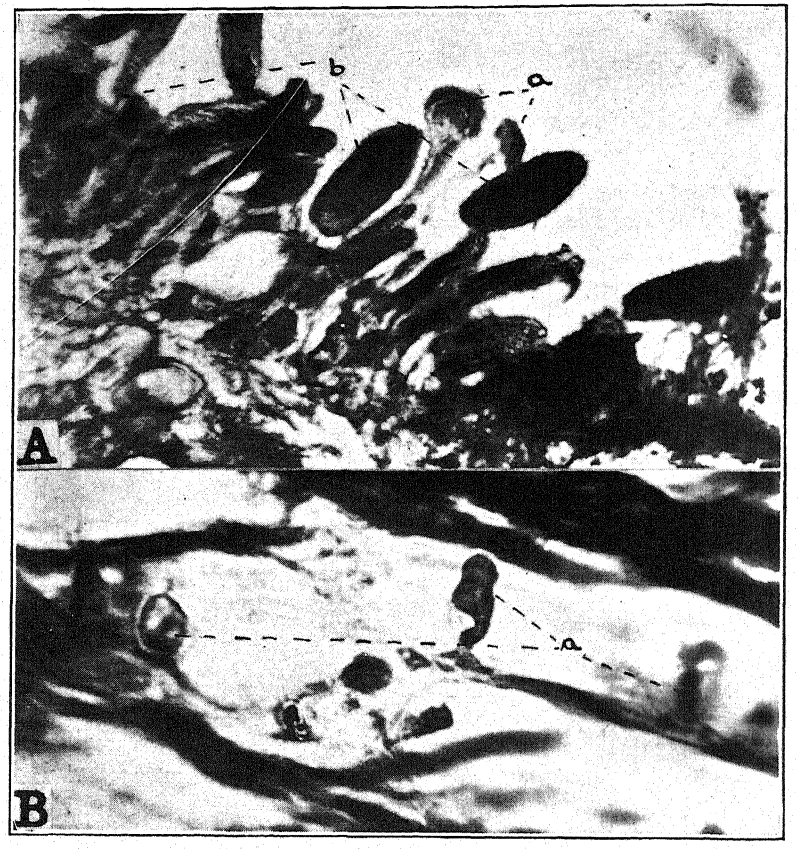


FIG. 3. Sections of diseased tissues. A. Portion of leaf sorus, X800. a, paraphyses; b, urediniospores. B. Section of hypodermis of fruit. a, haustoria of rust fungus.

being noninfected in the fall, spore pustules have been found early in the spring before the leaves unfold and at about the time when the buds are swelling. The fruiting bodies break through the bark at this stage without forming an apparent lesion. The epidermis of the host becomes raised slightly, the tissue directly adjacent being slightly water-soaked in appearance, and finally the urediniospores and paraphyses break through the bark. Later, as the twig grows in circumference, these areas split open lengthwise along the twig, allowing free egress of the spore masses. The lesions formed in the bark are superficial and localized and appear not to affect the host to any particular degree and in growing twigs soon are covered by new bark. In fruiting twigs, however, where the centripetal growth is not great, the lesions remain a menace in that a small percentage harbor a perennial mycelium. In these cases sporulation occurs again the following

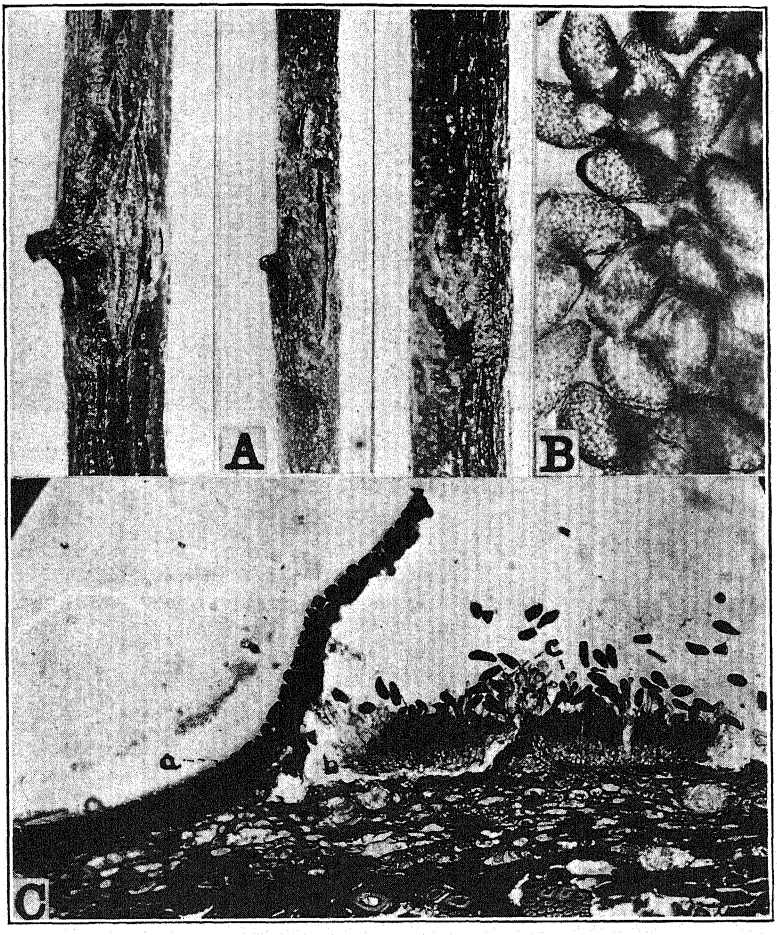


FIG. 4. A. Rust sori on bark of peach. B. Urediniospores. C. Section of bark sorus.

(second) spring along the margins of the old lesions. Microscopic examination of the affected regions reveals the fungus confined to the parenchyma of the cortex, the mycelium is intercellular, and haustoria are found within the parenchymatous cells. The fungus fruits by forming a stromatic layer of pseudoparenchyma just beneath the epidermis or bark of the host. From this there forms a mass of loose-woven hyphae upon which paraphyses and urediniospores are differentiated. The slight blistering or swelling of the surface of the host, observed at the point of pustulation, is not connected with an increase in the number of cortex cells but is due to the accumulation of the stromatic layer of the fungus below the epidermis. The paraphyses and urediniospores are similar to those of the leaf sori.

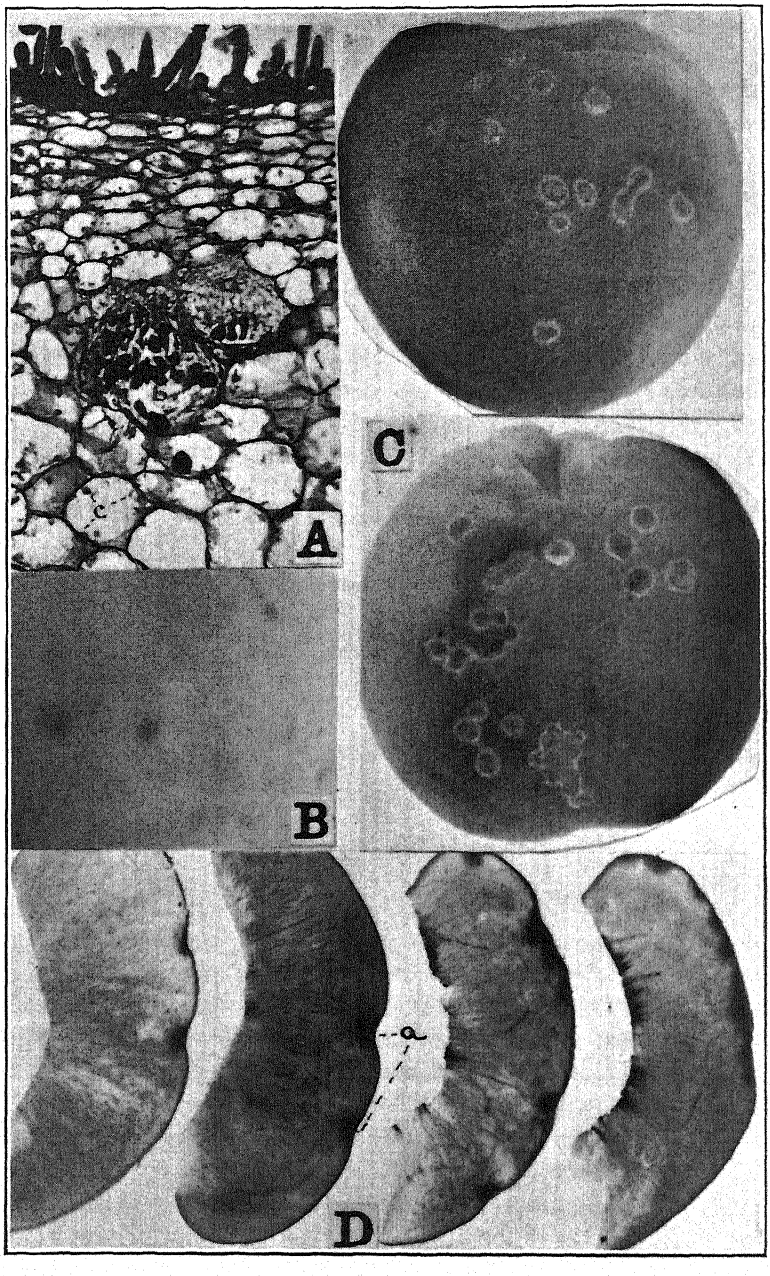


FIG. 5. Peach rust on fruit. A. Internal sorus. B. Mature fruit lesions. C. Young fruit infections. D. Cross sections of fruit lesions.

Fruit phase (Fig. 5). Fruit infection occurs only when germinating conditions are at an optimum for the fungus at a relatively late stage of fruit maturity. The areas affected appear first as water-soaked, dark green spots (Fig. 5, C). Growth is arrested, the spots become sunken as the adjacent tissue continues to put on new increment, and the central portions remain somewhat greener than the margins, becoming deep yellow when the lesions are older. On approaching maturity the normal pigmentation of the fruit occurs and the rust spots stand out vividly against this background. Urediniosori are formed in many, but not all, of the diseased areas on the fruit and these occur as dark brown, dusty areas in the very center of the region. In many cases, however, the sori never reach the surface, for they are formed within the hypodermis, sometimes covered with as many as 24 cell layers of this tissue, which, as the disease progresses, becomes tough and leathery but not killed. Figure 5, A, shows a typical case of submerged sorus in the fruit. The formation of these stromatic masses within the hypodermis and not, as in the leaf and bark, below the epidermal layer is no doubt a result of new increments being laid down by an active cambial region above the area originally affected.

The fungus mycelium in the fruit of the peach is intercellular and many haustoria are formed within the cells. At first the affected cells show no characteristic changes, but plasmolysis is noted later, the cells become collapsed, and a tough leathery region is formed which clings tenaciously to the adjacent normal tissue, causing, when the fruits are lye-dipped for peeling, a shallow-seated blemish which renders the fruit undesirable for canning (Fig. 5, D).

Aecial stage. Not observed.

Telial stage. Not found, either on peach or any related host in the Sacramento Valley.

EPIPHYTOLOGY

This rust, so far as determined, is confined in the Sacramento Valley region to varieties of clingstone and freestone peaches and has not been found on apricot, prune, plum, almond, cherry, or on any alternate host. The overwintering bark stage will be taken as the starting-point in the discussion of epiphytology. The observations cover a period of two entire years, with an additional one in which the rust had completed its development. This comprises the latter part of 1926 and all of the seasons of 1927 and 1928.

In the summer of 1926, after a survey of the various infected orchards as to orchard management, irrigation practices, presence of wind barriers, relation of infected orchards to the Feather River, and varietal susceptibility had been made, the amount and time of rainfall and the occurrence of

previous infection were indicated as the principal factors in the development of the disease. Methods of irrigation, as, for instance, flooding compared with furrowing (which appeared at first as being of some consequence), appeared not of any great concern in relation to continued infections. It was noticed that open ditches in which water continuously flowed slighly favored local infection but that this was of little practical importance.

The most important observation in this preliminary investigation was that of the bark phase of the rust which at this time, August, 1926, was found on the previous year's twigs but not on the current season's wood. As a result of this, a careful investigation was made as to the inception of this phase for it was early thought that this might furnish the means of overwintering of the fungus. From the manner in which successive periods of leaf infection occurred it appeared evident that such infections were correlated with rainy periods during late spring and early summer. At the same time, however, although many twigs were examined, no new bark infections could be found.

The winter of 1926–27 arrived with still no findings as to the formation of the new bark phase. Soon after the opening of the buds in the spring (1927) many leaf infections occurred and it was soon found that nearly all of these primary infections were adjacent to bark lesions which had recently appeared on the twigs of the previous season. In a few cases, where single lesions were found on leaves with no new bark lesions nearby, the infection appeared to be due to spores disseminated from old bark lesions, of which many were present on two-year-old wood.

In the fall of 1927 many twigs were labeled and examined throughout the winter and during the spring of 1928, with the result that two types of bark lesions were discovered—one that forms early in the winter and fruits early in the spring and another that sporulates later as the buds are swelling. The origin of this last type, which is by far the more common of the two, is not positively known but, judging from the twig observations just mentioned and indirectly from the results of spraying experiments, it apparently results from twig infections in the early fall, in which the fungus overwinters in the mycelial form. In the spring the fungus breaks out through the bark with groups of uredinia and the spores cause infection in the near-by leaves as they unfold.

This primary leaf infection, originating in bark lesions, is characterized by the infection of all the adjacent leaves and these are found below and above and to the sides of the bark lesion. Rarely does the infection spread more than two feet from the bark pustule. The leaves are usually heavily infected. Spore production in these primary foliage lesions occurs in from three to four weeks after the leaves come out, and, if conditions are favor-

able, secondary infections occur on other leaves as soon as the spores become mature. This crop of spores gives rise to further leaf infections and these are of importance to the grower, in that they are the source of the fruit infections and resultant loss of the crop.

As fall approaches, many of the leaf lesions that have remained dormant all summer become active again and sori are produced along their margins. As these sori mature and the fall rains come on, infections apparently take place in the bark of the current season's wood, which has thus far remained free of rust, and also occasionally in that of the two-year wood. These lie dormant throughout the winter and give rise to urediniospores in the spring, which are responsible for the leaf infection, and the life cycle begins anew.

Another and important phase of the bark infection was discovered during the spring of 1928. At this time, on trees that had received careful and timely sprays and showed no sori on the last season's twigs, leaf infections were encountered which had all the typical symptoms of being associated with bark pustules. Examination of two-year-old fruiting twigs revealed the presence of old bark pustules which had remained dormant during the summer and fall, and had become active at this time, giving rise to a new crop of urediniospores at the margin of the old lesions. This finding was of great importance in that it accounted for the observed tendency of the organism to escape treatment. This made the problem of eradication doubly hard, since it required not a single season but at least two for that purpose.

In summary, the fungus overwinters as urediniospores in the bark sori of the previous year, or as recent infections on bark, or by the renewed activity of old bark infections; the adjacent leaf surfaces are infected during the period following initial growth in the spring; subsequent infections occur on other leaves or on fruit but not on bark; the fungus ceases activity during the dry months of the summer; activity of the leaf lesions follows during the fall months and finally new infections occur on the bark of current season's wood during the fall, which remain dormant and invisible until spring. Several previous investigators have observed the bark phase of the disease on peach but they state, or leave the inference, that the infection takes place in summer along with that of the leaves, which was not the case in the Sacramento Valley during the period of this work.

EPIPHYTOLOGICAL FACTORS

Moisture relations, maturity and age of spores, temperature, and time are the factors of especial interest in the case of the peach-rust fungus, all playing important parts in the activities of the organism and its infection of the host. The effects of the above factors upon the germinability of the spores and upon the incidence of disease are included in the following

remarks. The order of their importance, judging from observations and experiments, is the same as that given above.

Relation to moisture. It became evident, after a detailed study of the disease as to its occurrence upon leaves, fruit, and bark and examination of precipitation records, that moisture played an important part in the life history of the organism in this district. Infrequent rains or rainy periods characterize the spring months in northern California, and in most years not a great deal of rain falls after the first week of April. The history of leaf infection in any one spring and early summer can easily be traced by the distribution of rusted leaves along the twigs. The usual case is to find most of the leaves affected close to the base of the twig. If the spring rains extend over a relatively long and late period, as they were in the spring of 1926 (Table 1), the leaves for some distance along the twigs become infected. It happened in this particular season that sufficient rain fell between May 4 and 9 to produce a period of late infection; the correlation could plainly be seen in the leaves.

There was no indication during this particular spring and summer, except in special locations, of another advance or period of infection. The epidemic during this season was severe and many orchards were almost defoliated and much fruit was damaged. Two very distinct rust attacks were noted in all orchards examined except one and that was located in the river bottom between large levees, where the top soil was moist each morning and heavy dews prevailed throughout April and May, causing a continuous but not severe infection on the leaves for a longer period than in the other orchards. It was apparent, however, even in this location, that fruit infection had not resulted from this continued leaf infection but was correlated with the heavy, continuous rainy period in May.

In 1927, the initial rust attack was light and very few orchards showed heavy early infestation. However, in the few orchards with which we experimented, the check trees showed a fair amount of rust. The season was two to three weeks later than the previous one and no sustained precipitation occurred. The primary rust had developed on the leaves surrounding bark pustules, but no secondary leaf infection was noted until the first week in June, when a large amount of leaf rust appeared. A rainy and cloudy period, in which the precipitation amounted to 0.12 inch, occurred during April 25 and 26, and this probably accounted for the secondary infection noted above. On May 27 and on June 7 considerable rain fell (0.82 and 0.67 inch). By the first of July many reports of infected fruit, widespread as to their sources, were received. There was an abundant secondary leaf infection in the affected orchards, evidenced by small pin-point rust spots on newer leaves together with the spotting of a great deal of fruit. It so happened that the rain of May 27 was irregularly distributed and

TABLE 1.—*Daily rainfall data of the months of September to June, inclusive, for the years 1925 to 1928, inclusive, at Marysville, California*[a]

1925–1926

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total
Sept.						.11																									0.11
Oct.											.02																				0.02
Nov.		.09	.06						.54	.15	.17	.20			.10														.21	.26	1.78
Dec.	.91																.69	.17													1.77
March																															.0
April			.30	2.89	.55	.20	.58	.25									.14														4.91
May				.20	.39	.18	.20																								0.97
June																															.0

1926–1927

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total
Sept.																				Tr.											Tr.
Oct.	1.20								1.28	.11																					2.59
Nov.										.29	.30	.24						.33	.75	1.70	.70		1.21	.26	1.00	1.12		.44			8.34
Dec.	Tr.	.24															.31			.15		.05			Tr.						0.75
March								.53	.09			.70	.79															.10		.62	2.83
April	.60		.14					.12																		.12					0.98
May																			.08	.04							.82				0.94
June							.66																								0.66

1927–1928

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total
Sept.																		Tr.													Tr.
Oct.																										.62				1.04	1.66
Nov.						.10	.60	.75	.75				.37							.43					.39						3.39
Dec.									.60			.05	.25											.95	.05			.17			2.07
March		.11		.25			.06	.32														.97	1.81	.56		.65					4.73
April	.20	.68									Tr.					Tr.	Tr.														0.88
May																															.0
June																															Tr.

[a] From climatological data of U. S. D. A. Weather Bureau.

one orchard with which we experimented was exposed only to a trace of rain. The June 7 rain, however, occurred with full force. This orchard had been sprayed immediately before this heavy rain (June 3–6). All the trees manifested severe leaf infection at the time the spray was applied. Nonsprayed check trees gave almost 100 per cent infected fruit, while the sprayed trees showed from 8 to 10 per cent, demonstrating the relation between fruit infection and the rain of June 7. In this case the evidence is completely in favor of the conclusion that rains are necessary for fruit infection. What had been supposed to be a mild rust season, through this single period of rainy weather, turned out to be an important one and brought the matter of moisture relation forcibly to the attention of the fruit growers.[2]

In the spring of 1928, when a heavy precipitation was noted in March (4.73 inches) and during the first two days of April (0.88 inch) considerable primary leaf infection occurred. No rain fell after April 2, and no secondary infections occurred on leaf or fruit. Not a single spotted fruit was observed during the spring or summer.

During these three years another period of infection occurred in the fall, when a great many leaves became rusted. During this time considerable dew or precipitated moisture accumulated at night in the orchards and, because of the shorter days and lower temperature, the time period for germination was increased. In other words, the fungus found optimum moisture conditions and, as a result, considerable leaf infection occurred.

The three seasons of observation strongly indicate that periods of considerable rainfall or dew accumulation are necessary for secondary infection of leaves and fruits. As to the relation of moisture to bark infections, it has already been noted that in the three seasons of our observations no bark infection occurred during the summer periods of leaf infection. It seems probable that rainy periods or prolonged periods of heavy dew are necessary, provided inoculum is present, for bark infection. It appears from the success of spraying experiments, in which early fall applications controlled this phase, that the bark infections occur in the fall and these are correlated with the above moisture periods.

Urediniospore germination (Fig. 6). As infection of the host is an aftermath of spore germination, considerable laboratory work was done in testing the relation of various conditions to the germinability of the spores of the parasite. A bulging of the spore wall is the first symptom of germination. A light-colored area in the cinnamon-brown spore membrane

[2] In 1930 a period of cool, humid, and rainy weather, ideal for the development of peach rust, occurred between April 23 and May 16. That the disease did not appear was apparently due to the lack of inoculum resulting from the absence of rust in 1928 and 1929.

becomes hyaline as the germ tube emerges. The wall of the germ tube appears always in continuity with that of the parent spore and at times the basal portion of the germ tube may be covered with the spines which so conspicuously mark that of the mother cell. The germ-tube wall is hyaline and, as a rule, constricted at the point of emergence. Sometimes this constriction may extend outward from the spore wall for some distance. (Fig. 6; b, j, and m). At other times it is confined to that portion near the germ pore. In a proper environment the germ tube grows rapidly, in one case at the rate of 9 μ in ten minutes, in another 13 μ in eight minutes. The tubes are hyaline and contain finely granulated protoplasm, more or less homogeneous throughout, with occasional spherical oil globules dispersed throughout the plasm. What appear to be nuclei are always located at the very tips of the growing hyphae. Branching occurs by budding and in no case have we found septa.

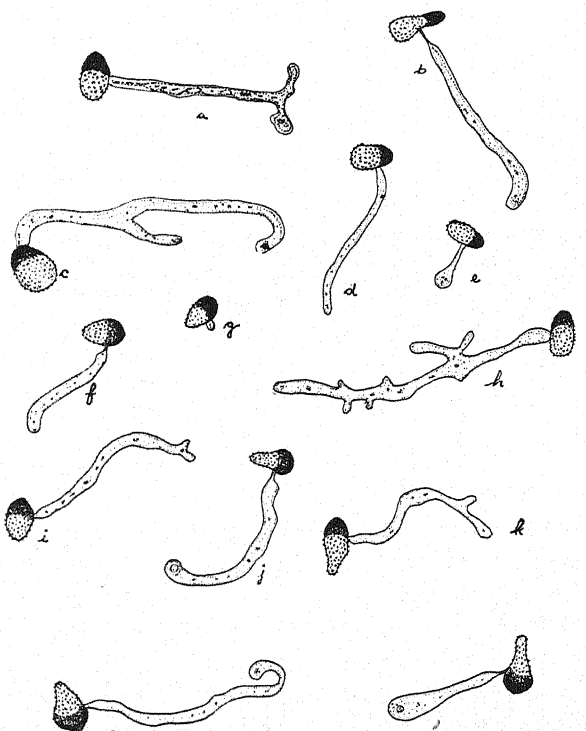


FIG. 6. Germinating urediniospores of the peach-rust fungus.

The phenomenon of branching apparently is associated with pressure and concentration, and this occurs after the apex of the germ tube has been arrested in its development. This allows a concentration of proto-

plasmic material with a resultant bulging at this point. If relief is not attained by budding, the swollen apex will burst and this appears to be the fate of many germ tubes in germination studies. If growth is continuous germ tubes reach considerable lengths, some over 700 μ having been observed.

Relation of humidity to germination of urediniospores. The question of the moisture condition necessary for infection in the field has been shown to be correlated with reasonably long periods of saturated atmospheric conditions, such a period being one in which a film of precipitated moisture is present on the under surface of the leaves. In the laboratory such a condition is easily reproduced and can be obtained either by placing the spores directly in contact with a film of water or by placing them in a saturated atmosphere, such as is produced in a closed chamber. It was found that the latter method furnished a maximum oxygen tension without which spores appeared not to germinate unless in a stage of complete maturity. It was noticeable in our germination experiments, especially those of 1926 in which relatively old material was used, that the germination of spores on water drops was correlated with their age and that the germinability of these same spores, when placed directly in a saturated atmosphere in which there was a maximum oxygen tension, was considerably greater. Keitt and Jones (26) reported the same phenomenon as occurring with the conidia of *Venturia inaequalis* (Cooke) Aderh. Allen (1) reported in the case of the urediniospores of *Puccinia glumarum* (Schm.) Eriks. and Henn. that rain water and not tap or distilled water favored germination. This may have been due to oxygen tension.

Considerable data relative to the effect of moisture on the germinability of urediniospores occurs in the literature. The differences between species in this relation appears to be slight and it is the general opinion that films of precipitated moisture are necessary for germination. The works of Melhus and Durrell (30), Peltier (31), and Duggar (19) are particularly suggestive in showing the importance of this factor in the life cycles of rusts. The present findings in the case of the urediniospores of the peach-rust fungus are that either a saturated atmosphere or a film of precipitated moisture is ideal for germination. When the spores are immersed in distilled water germination is arrested; but, when floated on water drops, germination proceeds within three hours if the spores are mature. Table 2 shows the relation of relative humidity to germination. It should be clearly understood, however, that it is quite difficult to draw a distinction between films of moisture, such as one uses in hanging drops, and those which must occur when a saturated atmosphere is used. In the latter case the film envelops the spore but in the former the spore appears to float on the surface, being in contact with the film at one point. It is true, how-

ever, according to Peltier (31) and Melhus and Durrell (30) that there is a difference, as they reported no germination in saturated atmosphere for grain rusts. It is not clear what differences are responsible for this, but possibly the nature of the spore wall and especially its wax content may influence this phenomenon. Melhus and Durrell show that paraffin wax was favorable to an increase in germination and we have demonstrated, as shown in table 13, that light oils stimulate germination even so far as to allow spores to germinate when the untreated ones did not.

TABLE 2.—*Effect of relative humidity upon germinability of urediniospores of peach-rust fungus. 22° C. for 12 hours*

Relative humidity	H_2SO_4 specific gravity	H_2SO_4 percentage	Spores counted	Spores germinated
1.5	1.754	82.0	2420	0.0
10.5	1.569	66.0	1879	0.0
18.5	1.503	60.0	2321	0.0
42.0	1.380	48.0	3287	0.0
58.3	1.300	39.0	2578	0.0
70.4	1.250	33.4	3168	0.0
85.7	1.170	23.5	2988	0.0
95.0	1.090	13.0	3560	0.0
97.5	1.050	7.37	4267	0.0
98.7	1.030	4.49	3897	0.0
99.1	1.020	3.03	4124	0.0
99.5	1.010	1.57	3800	0.0
100.0	1.000	0.00	4688	1988.0

Discussion of moisture relations. Extensive peach-rust infections throughout the three years of observation were clearly connected with rainy periods or with situations having heavy and continuous dews and not with periods of irrigation.

If irrigation practices were responsible for differences in infection this was not noticeable in the places examined, as just as much rust occurred in nonirrigated orchards as in furrowed and flooded situations. Relative to the last point it is interesting to note that there have been certain cases in which secondary infections have resulted on leaves attached to low-lying branches adjacent to open ditches or where, because of faulty drainage, accumulations of irrigation water occur. In these instances we are of the opinion that the cumulative effect of many saturation periods favored germination. Probably, during this time, a certain percentage of spores germinated and caused leaf infections. In any case this type of infection is not severe and the lesions occur on only a few leaves. That irrigation is

not responsible for fruit infection was clearly demonstrated during the 1928 season, as no fruit rust occurred in any of the situations referred to above; in fact, no secondary rust on leaves occurred, except in the above special cases. Irrigation during times of rainfall might cause some increase in the length of the saturation period and thus have a direct influence on infection.

Laboratory studies demonstrate that precipitated moisture is essential for germination of the urediniospores and supplement the observations in the field.

Relation of age to germination of urediniospores. The age and maturity of urediniospores constitute an important factor in their capacity to germinate. The viability of urediniospores, in general, is of relatively short duration and, because of this, considerable mystery surrounds the survival of many of the species having only this form in their life cycles. In this respect, Barclay (6) was able to germinate urediniospores of *Puccinia coronata* var. *himalensis* for 4 to 5 months; Melhus and Durrell (30) found that those of *P. coronata* persisted for at least 55 days but that the temperature at which the spores were held constituted an important factor. At room temperature they remained viable only 30 days. Maneval (29) reviewed the literature relative to this and table 3 gives a synopsis of various findings. That various differences are noted, even with the same species, is due in part to the method of collection, age of material, temperature at which held, and the condition of host plants.

TABLE 3.—*Longevity of urediniospores of various species of rusts, after Maneval*

Author	Fungus	Longevity
		Days
Ward	*Puccinia dispersa* Eriks.	61
Fromme	*Puccinia coronifera* Kleb.	84
Reed and Holmes	*Puccina coronata* Cda.	77 (91?)
Melhus and Durrell	" "	55
Peltier	*Puccinia graminis* Pers., form 3	92
Frazier	*Puccinia helianthi* Schw.	182
Spaulding	*Cronartium ribicola* F. de Wal.	155[a]
Taylor	" "	Overwintered
Maneval	*Uromyces striatus* Schr.	176[a]
"	*Puccinia sorghi* Schw.	158[a]
"	*Puccinia coronata* Cda.	157[a]
"	*Puccinia menthae* Pers. var. *americana* Burr.	173
"	*Uromyces caryophyllinus* (Schr.) Wint.	185
"	*Puccinia amorphae* Curt.	89

[a] Average.

The viability of the urediniospores of the fungus under study is markedly variable and is greatly influenced by the source and the environmental conditions affecting the host at the particular time of collection. Peltier (31) has shown that the humidity at which urediniospores are held influences the period of viability. It was our experience that viable spores were obtained for a longer period from leaves remaining on the host than from material which had been removed from the trees, but this may have been due to the production of successive generations in the sori on the tree. In the case of bark pustules, many have been tested, and from these even late in the fall after the leaves have fallen a small percentage of viable urediniospores can be recovered. In the case of the bark sori some were wide open, due to growth of the host, while others were entirely submerged and covered with bark tissue, but the percentage of spore germination remained the same in both cases.

While it is interesting to know that mature urediniospores of this fungus overwinter to a slight degree in the bark pustules, this is not actually of great importance in the life history, since very little infection occurs from this source. The vast amount of urediniospores that develop in the leaf sori during the primary, secondary, and tertiary infections of the spring and early summer months is the important source of supply, and it is the relation of these urediniospores to the life cycle and the effect of maturity on them that are of interest to us in this respect.

Relatively speaking, the urediniospores of the leaf sori are short-lived. The continued recurrence of new sori in seasons of unusual summer rains, however, bridges these short cycles and to all intents and purposes longevity is not important in these years. It is during a relatively dry summer season that this factor is important, not so much in the life cycle, but economically. Table 4 shows the absolute values for certain sets of experiments. Urediniospores collected at intervals from diseased leaves on the tree of a known early infection period contained viable spores over a period of 6 weeks. In a later infection occurring in June, this period was shortened to 4 weeks. Urediniospores which remained attached to detached leaves showed 56 per cent germination when first tested, 29.8 per cent after 22 days, and, after 33 days, no germination (Table 5).
At no time did they show a larger percentage than at the beginning. Urediniospores detached from living leaves 17 days after collection gave 39 per cent germination, about the same value as that of spores from detached leaves. These values are only relative, since other factors are important in determining the number of viable spores one may find. Melhus and Durrell (30) have shown that urediniospores may, if detached from their sori, show an increased percentage of germination due to the after ripening of spores. The results obtained in germination experiments

TABLE 4.—*Longevity of urediniospores of peach-rust fungus in sori on living leaves upon the tree*

Date collected	Source		Age of (weeks)			Germinability		Per cent germinated
	Bark	Leaf	Bark	Leaf	Sorus	Spores counted	Spores germinated	
4–19–27		"		6	1	602	none	...
4–20–27		"		6	1	1280	none	...
4–28–27		"		7	2	3138	39	1.2
5– 6–27		"		8	3	1281	63	5.0
5–17–27		"		10	5	2516	387	15.4
5–24–27		"		11	6	1293	486	37.7
5–28–27		"		11.5	6.5	4738	3118	67.2
6–24–27		"		15.4	10.4	3673	38	1.0
7–28–27		"		20.2	15.2	1013	150	1.4
4–19–27	"		?			3280	1388	42.3
4–28–27	"		?			1586	879	55.4
5– 2–27		"		7.5	1.5	1081	2	Tr.
5– 9–27		"		8.0	2.0	3266	38	1.1
5–17–27		"		10.0	4.0	1281	16	12.5
5–19–27		"		10.0	4.0	2520	34	13.5
5–28–27		"		11.5	5.5	2096	553	26.8
6–22–27[a]				15.0	2.0	1672	10	.6
7– 6–27[a]				17.0	4.5	414	145	35.0
7–14–27[a]				18.0	5.0	616	282	45.0
7–28–27[a]				20.0	7.0	931	407	43.7
8–13–27[a]				22.5	9.5	680	14	2.0
6–22–28[b]		"		?	5.0	562	170	30.3
6–22–28[b]		"		?	5.0	508	193	38.0
6–20–28[b]		"		?	1.0	482	2	0.4
6–27–28[b]		"		?	2.0	931	106	10.2
7– 9–28[b]		"		?	4.3	586	393	67.7
7–14–28[b]		"		?	5.0	632	287	45.4
7–28–28[b]		"		?	6.0	581	30	5.1
7–11–28[b]		"		11.5	9.5	1664	54	3.2
7–11–28	"		11.5		11.5	931	75	8.0
9–21–28	"		17.2		17.2	1336	27	2.0
10– 8–28	"		20.0		20.0	1491	25	1.7

[a] New infections.

[b] New sori from old infections.

depend a great deal upon the manner in which the urediniospores are obtained from the sori. In any sorus at any given age there will be a difference between mature and immature spores. In most of the literature no reference is made as to the method of gathering the spores. Melhus and Durrell state that it made no difference whether the spores were shaken

TABLE 5.—*Longevity of urediniospores of peach-rust fungus on detached leaves, at 20–22° C.*

Date collected	Date tested	Spores counted	Spores germinated	Per cent germinated
9–29–27	9–29–27	5381	3013	56.0
9–29–27	10– 4–27	3669	1815	49.5
9–29–27	10–14–27	1762	705	40.0
9–29–27	10–21–27	3021	900	29.8
9–29–27	11– 2–27	927	0	0.0
10– 6–27	10–16–27	4638	1809	39.0

or whether they were scraped from the sori. In our experiments the spores first shaken off, presumably the oldest, gave the highest percentage of germination; those shaken off later germinated less; and those finally scraped off germinated not at all. Doran's (17) method of giving the percentage of germination at optimum as 100 per cent and raising the other percentages accordingly is satisfactory for any given sorus but it is obvious that each may have a different percentage even at the optimum. Regardless of method, in the case of urediniospores, only relative values are realized, as each source of material is different from the next. General trends can be arrived at through the accumulation of mass data, but it is obvious that absolute values are impossible unless the sori have a known history.

Longevity of spores becomes important in the life history of the rust fungus in that it operates to limit secondary and tertiary infection. Secondary infections are important not only in continuing the life history but also in determining the economic losses due to fruit infections. If conditions which favor secondary infection are absent, the further development of the fungus is checked by the death of the comparatively short-lived spores produced in the primary infection. Tertiary infections operate to carry the fungus into the fall of the year. It is obvious that in case an exceptionally abnormal fall season occurred in which rain was absent, this factor would be of the greatest importance, in that the fall infections of the wood would be reduced and overwintering would be more or less restricted to the older lesions.[3]

Relation of temperature to germination of urediniospores. The rust fungi in general have wide ranges of temperature at which the spores germinate. Doran (17) tested several species (Table 6) and found that

[3] This apparently happened in the season of 1929–30, when no rain fell until December 10.

TABLE 6.—*Spore germination temperatures of various rust fungi, after Doran*

Fungus	Temperature: Degrees C.		
	Minimum	Optimum	Maximum
Aeciospores of *Cronartium ribicola* F. de Wal.....	5	12	19
Urediniospores of *Cronartium ribicola* F. de Wal.	8	14	25
Aeciospores of *Gymnosporangium clavipes* C. and P.	9	16	29
Urediniospores of *Puccinia antirrhini* Diet. and Holw.	5	10	20
Puccinia malvacearum Mont.	3	14	30
Urediniospores of *Uromyces caryophyllinus* (Schr.) Wint.	4	14	29

TABLE 7.—*Effect of temperature on the germinability of urediniospores of the peach-rust fungus. 12 hours or less*

Temperature, degrees C.	Spores counted	Spores germinated	Per cent germinated
1	3798	0	0.0
7	4296	0	0.0
8	2242	7	.31
10	1610	436	27.0
12	1561	436	28.0
14	3753	1554	41.1
15	2012	946	47.0
16	3637	1320	36.5
21	3762	2025	53.8
22	4320	1490	34.5
23	4750	2000	42.1
24	4710	2270	48.2
25	5388	2220	41.2
26	5640	1740	30.9
27	3816	584	15.3
27.5	4000	620	15.0
28	3340	672	19.7
29	4032	976	24.0
30	3915	684	17.4
31	3456	464	13.4
32	3496	744	21.0
33	3856	240	6.2
34	3600	64	1.9
36	4104	80	1.9
37	4104	64	1.5
38	3554	48	1.3
39	1872	0	0.0
40	4896	0	0.0

relatively low temperatures favored many rust urediniospores. Peltier (31) showed that high temperature lowered the percentage of germinating spores, while low temperatures favored it. Walker (37) pointed out, in the case of the smut fungus, *Urocystis cepulae* Frost, that optimum temperatures for chlamydospore germination also coincided with rapidity of length growth. Doran points out "that although urediniospores germinate over a wide range of temperature, there is a material degree of host infection only when the temperature is near the optimum."

The urediniospores of the peach-rust fungus have a wide range of germination temperatures (Table 7), the minimum being 8° C. and the maximum 38° C. with the optimum between 13° C. and 26° C. Melhus and Durrell (30) consider the length growth of the germ tubes as an indication of optimum. We have no comparative information relative to this for the peach-rust fungus, but, at 22°–24° C., the germ tubes appear to continue their growth at the maximum rate without injury. Below 14° C. and above 26° C. the germ tubes appear to be injured. At high temperatures the tips of the germ tubes burst soon after emergence, while others show branching. At low temperatures bursting does not occur but frequent branching is the rule, the germ tubes exhibiting all sorts of convolutions in contrast to the even, continuous growth at 22°–24° C. The temperature curve of germination (Fig. 7) appears to have a fairly rapid rise, after which it flattens out for some distance and drops off rapidly at first, then finally goes into a gentle slope. This is, in general, the trend of germination. Some discrepancies occur, but this is to be expected, since spore material is so variable. The field temperatures during the early season when rust attacks begin correspond, in general, to that part of the curve during which the period of optimum germination occurs. High temperatures are injurious to spores in that those kept at 40° C. and over for more than 12 hours, when removed to a suitable environment, never germinate. In the field, however, urediniospores seldom are subjected to such a high temperature for any length of time.

Where a fungus has such a wide latitude of favorable temperatures for germination, provided other conditions are at optimum, it seems logical that this factor would not be of great importance in natural infection. It is pointed out, however, by other workers that periods of infection coincide with the optimum germination temperature period. If this is so, a wide range of germination might, in the case of temperatures of either extreme being prevalent for any length of time, actually be effective in a control of the fungus, in that germination would result without infection, and thereby reduce the amount of inoculum.

Effect of fungicides on the germination of urediniospores. The literature on the subject of the control of peach rust, although scanty, indicates that Bordeaux mixture and lime-sulphur sprays may be used to advantage.

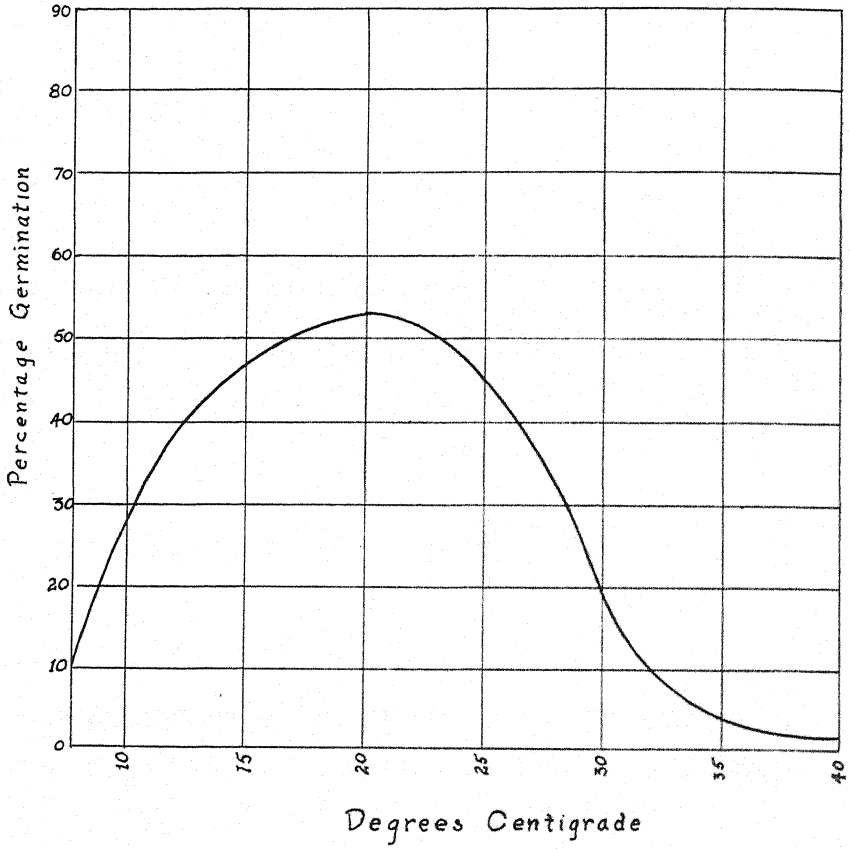


FIG. 7. Effect of temperature on germinability of urediniospores of peach-rust fungus. Twelve hours or less.

Galloway (23) reported the effectiveness of Bordeaux mixture in controlling plum leaf rust (*Puccinia pruni-spinosae* Pers.). He stated that Professor T. L. Brunke, at his suggestion, conducted a series of experiments in 1888 at the Texas Agricultural College with the view of finding a remedy for this disease. Brunke found that spraying with Bordeaux mixture in August gave good control. Those trees which were not treated lost nearly all their foliage, while those sprayed had lost only a very small percentage.

Pierce (32) reported, on the basis of one year's experiments, that Bordeaux mixture and other copper compounds applied at the pink stage of the bloom were of value in control of prune rust.

Fairchild (22) noted that, if the conclusions of Scribner and Pierce are well grounded, this is the first case to be established of a rust yielding to Bordeaux mixture.

Cunningham (15) advised the use of lime-sulphur sprays.

McCalman (28) reported that lime sulphur 1–50 or an application of Bordeaux mixture (2–2–40) kept down the disease.

Doran (17), testing urediniospores of various rust fungi, showed that these, as a rule, were very resistant to copper. Butler (12) and Doran (loc. cit.) found that dusting sulphur controlled Antirrhinum rust, and Smith (34, 35) showed that this material was effective against the asparagus rust in California. The Uredinales, in general, are, from all indications, comparatively resistant to copper and susceptible to sulphur compounds.

TABLE 8.—*Effect of copper sulphate on urediniospores of peach-rust fungus. 22° C. 12 hours*

Dilution percentage	Spores counted	Spores germinated	Per cent germinated
2.0	982	0	0
1.0	932	1	.1
0.5	868	0	0
0.25	943	0	0
0.125	841	41	5.0
0.06	531	40	7.5
0.03	1154	104	9.0
Water	2664	447	16.5

TABLE 9.—*Effect of various copper mixtures on urediniospores of peach-rust fungus at 22 C.*

Kind of dilution	Spores counted	Spores germinated	Per cent germinated
Homemade Bordeaux 4–4–20	1286	145	11.3
Homemade Bordeaux 2–2–50	1469	241	16.4
Homemade Bordeaux 5–5–50	6084	747	12.3
Homemade Bordeaux 8–8–50	1956	124	6.3
Commercial Bordeaux Ortho Brand 1–40	3463	1282	36.8
Commercial Bordeaux Ortho Brand 1–80	371	52	14.3
Borco Winter str.[a]	1710	408	22.9
Copper sulphate	1437	0	0.0
Water	6291	2206	36.0

[a] Ammoniacal copper compound.

Copper compounds. Table 8 shows the effect of copper sulphate on the urediniospores of the peach-rust fungus and table 9 the effect of various other copper compounds. It can readily be seen that the fungus is relatively resistant to copper. In field experiments, however, this was not so marked, and, although spores fully covered with Bordeaux mixture germinate when brought into the laboratory, there appears to be a distinct decrease in the amount of leaf infection in trees sprayed with this material as compared with unsprayed trees. The tests in the laboratory with spores treated with Bordeaux mixture show a considerable amount of germination, but this percentage is always below that of the checks. On microscopical examination one finds the spores along the edge of heavy Bordeaux mixture spots germinating first, and this is true also of those spores lying in the sparsely covered regions. The spores heavily covered with the colloidal material germinate last or not at all. On slides kept more than three days,

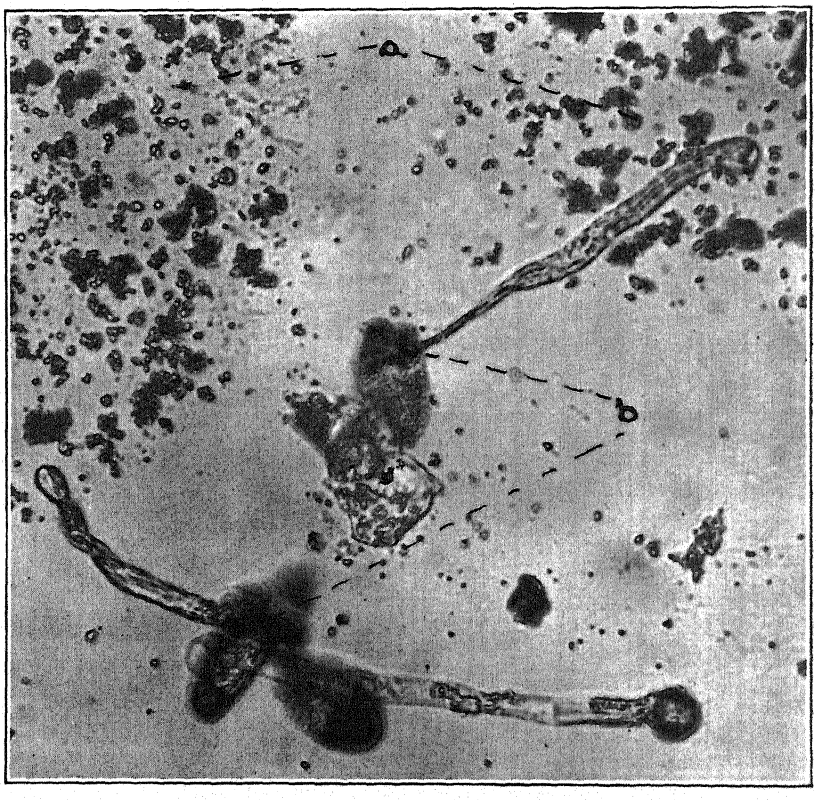


FIG. 8. Urediniospores of peach-rust fungus germinating in a field of commercial Bordeaux mixture. Material sprayed upon spores on glass slide before germination.

the spores showed germination even in the heavily coated parts. Figure 8 shows germinating urediniospores in a field of commercial Bordeaux mixture of winter strength. This is not an unusual finding and is a common phenomenon with homemade and commercial Bordeaux mixtures and for ammoniacal copper solutions, as well.

Polysulphides. Polysulphides, as in commercial lime-sulphur solution, are very toxic to the urediniospores of this fungus, even in highly diluted solutions (Table 10). The effect on the spores and germ tubes, according to Goldsworthy (24), is direct, the sulphide reducing the protoplasm, which causes the death of the organism (Table 11). Figures 9 and 10 show the lime-sulphur-treated spores and germ tubes.

TABLE 10.—*Effect of lime-sulphur and powdered sulphur on urediniospores of peach-rust fungus at 22° C.*

Kind and dilution	Spores counted	Spores germinated	Per cent germinated
Commercial dry-lime-sulphur 14–100	4036	242	6.0
Liquid-lime-sulphur 1–100	1376	12	0.9
Liquid-lime-sulphur 1–50	3001	0	0.0
Liquid-lime-sulphur 1–10	3378	0	0.0
Powdered sulphur old lot	2060	198	9.60
Powdered sulphur new lot	8336	118	1.40
Sulphur-paste gas residue in water	1723	49	2.80
Water	12425	3516	28.30

Commercial dry-lime-sulphur (Sherwin-Williams) and sodium sulphide compounds are highly toxic to the urediniospores. In recent years liquid-lime-sulphur solutions have caused considerable burning to both foliage and wood of the peach, and the growers are becoming alarmed over the situation and are turning again to the use of copper compounds.

Powdered sulphur. Powdered sulphur has been extensively used in the control of fungi, especially powdery mildews and rusts. This material has been used against the peach-rust fungus by many growers, and the success attained has been difficult to determine, due to the varied conditions under which it was used. Sulphur apparently acts best at high temperatures and in still air and the lack of success of many growers may be attributed to

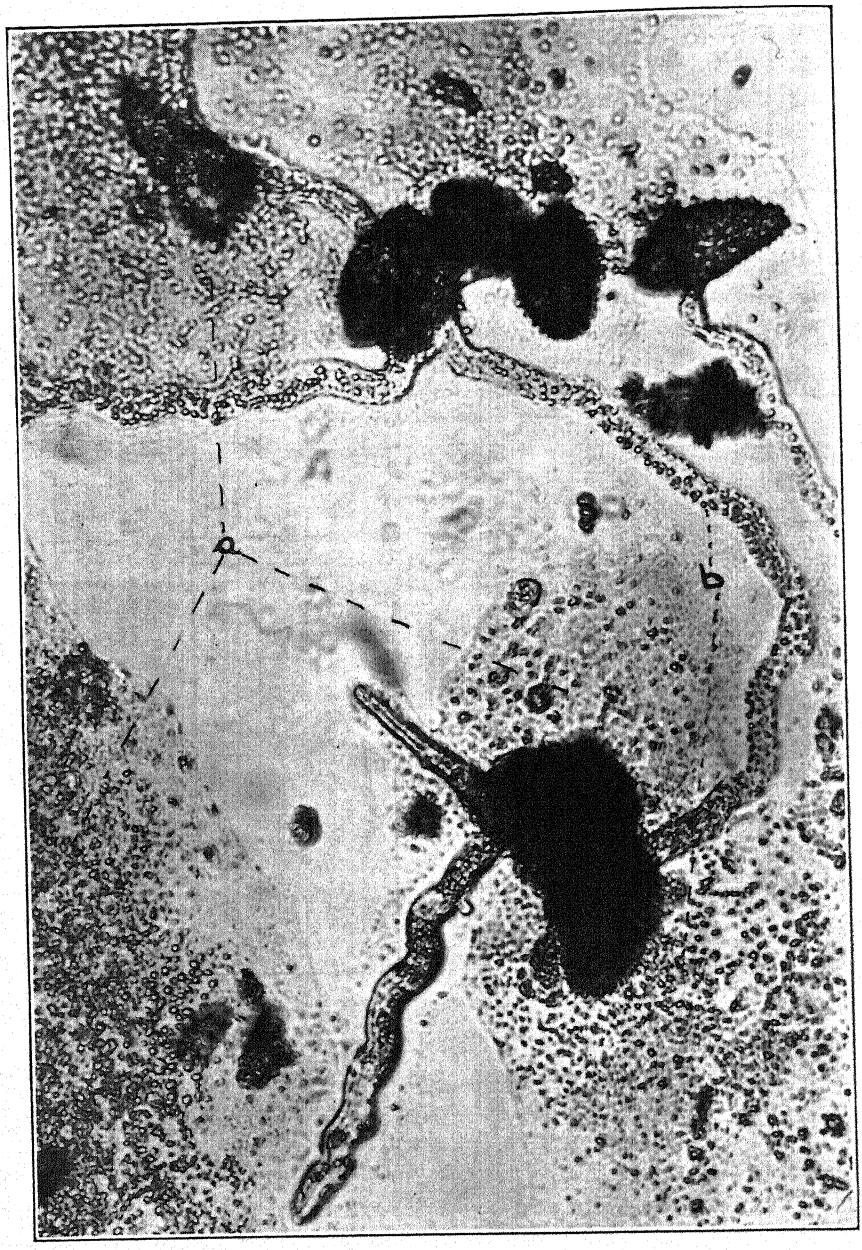


FIG. 9. Germinating urediniospores of peach-rust fungus sprayed with liquid-lime-sulphur 1–50. Sulphur particles on slide and in germ tubes.

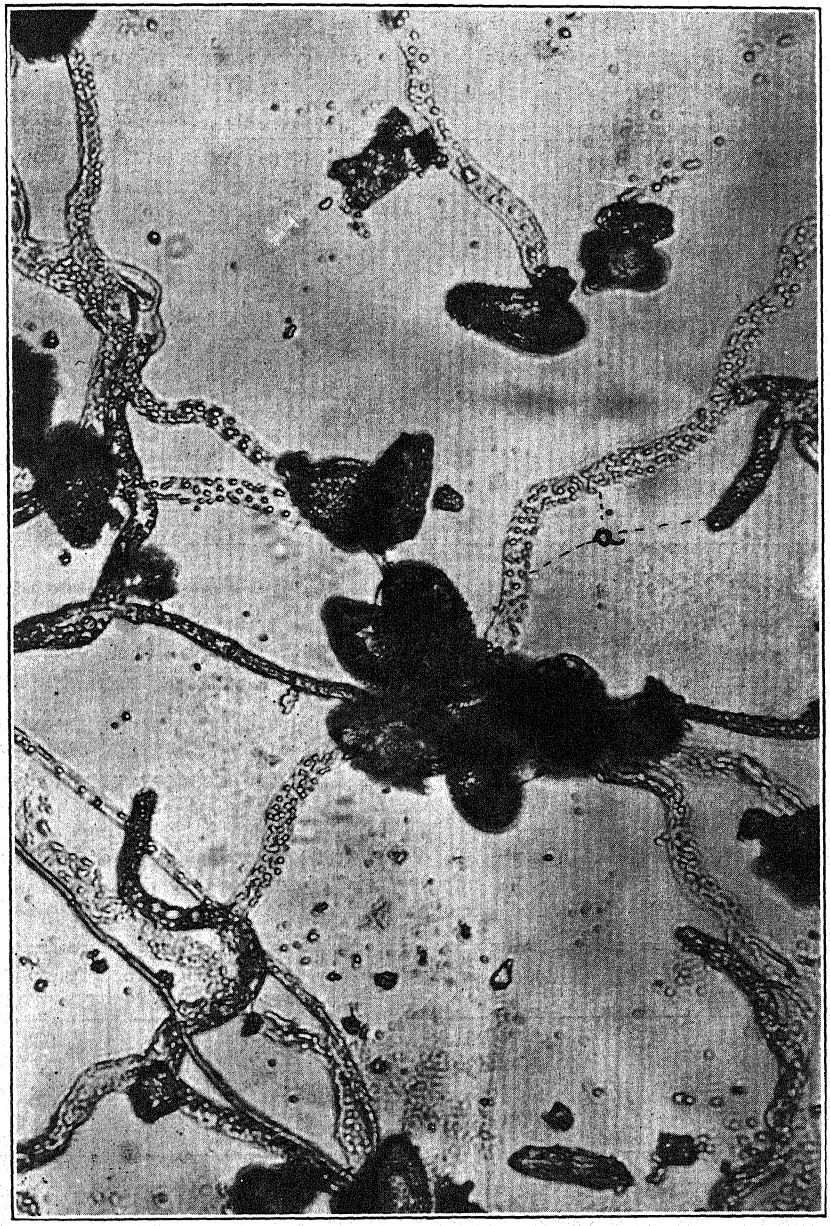


FIG. 10. Germinating urediniospores of peach-rust fungus sprayed with liquid-lime-sulphur 1–50. Sulphur particles removed from slide but retained in germ tubes.

TABLE 11.—*Effect of liquid-lime-sulphur 1–10 on the growth of germ tubes of the urediniospores of the peach-rust fungus at 22° C.*

Germ tube	Time sprayed	Length of germ tube (microns)			
		3 hours	6 hours	9 hours	12 hours
1	third hour	6	6	6	6
2	"	8	8	8	8
3	"	6	6	6	6
4	sixth hour	–	17.5	17.5	17.5
5	"	–	23.0	23.0	23.0
6	"	–	15.0	15.0	15.0
7	ninth hour	–	–	38.0	38.0
8	"	–	–	46.5	46.5
9	"	–	–	35.0	35.0
10	twelve hours not sprayed	–	–	–	61.5
11	"	–	–	–	82.0
12	"	–	–	–	77.0
13	twelve hours average of 12 tubes. Not sprayed	—	—	—	71.5

the fact that the temperature was not high enough at the time of application. With laboratory experiments, sulphur dust and a liquid mixture of a gas-house residue "sulphur paste" gave excellent results (Table 10). The sulphur dust and the paste were applied to spores on slides and allowed to germinate under the optimum moisture and temperature constants. In one case careful observations made during the 1928 season of peach trees dusted during June showed that an excellent control was effected (Table 12).

TABLE 12.—*Effect of powdered sulphur (orchard tests) on urediniospores of peach-rust fungus. Spores removed from leaf sori after trees were dusted and tested in moist chamber at 22° C.*

Date of applying sulphur to trees	Date of testing spores	Spores counted	Spores germinated	Per cent germinated
6–5–28	6–15–28	5658	14	0.2
6–5–28	6–17–28	4822	120	2.7
Check	6–20–28	631	50	8.0
6–5–28	6–21–28	5328	60	1.1

Other fungicides. The successful use of sodium silicofluoride, reported by Anderson (2) in the control of *Bacterium pruni,* the control of green storage mold of citrus fruits by the use of borax solutions, and the increasing use of mercury compounds as well as light oil sprays in the control of fungi prompted their use in laboratory tests. Table 13 shows the effect of

the various materials tested. In general, none of these, with the single exception of sodium silicofluoride, were promising and the light oil Volck stimulated germination. It is becoming the practice, since oils of various grades are coming into use for winter treatments, to combine various substances, such as Bordeaux mixture and sulphides, with them. For this reason we tried a mixture of sodium sulphide and a light oil emulsion in the proportion of 0.5 per cent sodium sulphide to 3 per cent oil emulsion in 1,000 cc. of water. A solution containing 2 per cent liquid-lime-sulphur and 4 per cent oil emulsion was also used. Almost complete inhibition of spore germination was effected in the laboratory but, in the field, these sprays were not so successful.

Sodium silicofluoride 0.25 per cent was used in field experiments but the injury to the foliage was so pronounced that its use was discontinued.

TABLE 13.—*Effect of various fungicides on urediniospores of peach-rust fungus at 22° C.*

Kind and dilution	Spores counted	Spores germinated	Per cent germinated
Sodium silico-fluoride 0.25%	5529	25	0.4
Borax solution 2.5%	496	22	4.5
Uspulun 0.25%	1951	120	6.2
Semesan 0.25%	1850	132	7.1
Volck oil emulsion[a] 3.0%	3560	1226	34.4
Lime-sulphur 2%, oil 4%	4433	57	1.3
Sodium polysulphide 5 gms.–30 cc. oil emulsion in 1000 cc. H_2O	1350	0	0.0
Water	12425	3516	28.30

[a] Apparently stimulated germination.

IDENTITY OF THE PEACH-RUST FUNGUS

The peach-rust fungus is commonly assumed to be identical with the well-known prune rust, *Tranzschelia punctata* (Pers.) Arth. (*Puccinia pruni-spinosae* Pers., *P. pruni* Pers.). This fungus has been reported in California for many years upon prune, plum, apricot, and almond, as well as upon the peach. (Scribner (33), Cooper (14), Pierce (32), Smith and Smith (36), Barrett (7, 8, 9), Blasdale (10).) A peculiar feature, particularly marked in the present instance of this peach-rust epiphytotic, is the varying specificity of the parasite as to host in different localities and the variation in the occurrence of the different spore forms on the various hosts and in different places. During the present work in the Sacramento Valley (1926, 1927, and 1928), considerable attention was paid to this phase of the subject. During this time the rust was never found in that

region on any host except peach. Even though prune trees stood immediately adjacent to badly rusted peaches no trace of the disease was found in the former host in any case examined. Regarding spore forms, none but urediniospores was ever found on peach trees in the Sacramento Valley, and many thousands of leaves and sori were examined.

In the Santa Clara Valley, south of San Francisco Bay, a very different situation exists relative to what is supposedly the same fungus. In this district, where prunes, plums, peaches, and apricots are extensively planted, rust develops late in the season almost every year upon the leaves of nearly all the prune and *domestica* plum trees. In this case the fruiting bodies, so far as determined, are all teliospores. Rust is never conspicuous upon peach or apricot and, during the years of the investigation herein reported considerable attention was paid to this point. Many cases were examined of peach and apricot orchards directly adjacent to prune orchards where the lower side of every leaf of the latter was covered with teliosori of the rust. Not a single pustule was found on peach or apricot. This reversal of conditions as to the comparative occurrence of rust on peach and prune in the Sacramento and Santa Clara valleys is most complete and striking, together with the predominant, if not exclusive, development of urediniospores on the peach in the Sacramento Valley and teliospores on prune and plum in the Santa Clara Valley. On apricot no rust was found in either of these districts during the period of this work. In 1927, however, a severe outbreak of apricot rust occurred at Arroyo Grande, a small valley opening directly on and within one or two miles of the ocean with a very cool and humid summer climate. This is in San Luis Obispo County at a point about midway between San Francisco and San Diego. Such an outbreak is not an uncommon occurrence in this locality. The rust develops on foliage and fruit during spring and summer with both urediniospores and teliospores.

Turning to the literature, Scribner (33) reports this rust fungus from California and other States on cherry, peach, apricot, and plum, and states that only the uredinial stage occurs on peach, while on the plum urediniospores may or may not be present, but there are sometimes a few mingled with the teliospores. Cooper's (14) publication is a repetition of that of Scribner. Pierce (32) amends the statement of Scribner regarding spore forms on the peach, stating that "The spores are mostly uredospores, although the teleutospores are often found at least in California." . . . "In the Annual Report of the Commissioner of Agriculture for 1887, pp. 353–354, it is said that no teleutospores are developed on the peach. Although less abundant than the uredo form, they have invariably been found in badly infected peach leaves in southern California, and these leaves have

been obtained from widely separated points in Los Angeles and Orange counties. They were fully matured by the middle of October." Smith and Smith (36) report the rust on almond, peach, plum, and prune. Barrett (7, 8, 9), who studied the disease in southern California, found a limited production of teliospores on the peach. Blasdale (10) states: "Uredinia and more rarely telia on cultivated peach, plum, prune, almond, and apricot, especially in the southern part of the state, but widely distributed." Many writers elsewhere, particularly in Australia, have noted the comparative scarcity of teliospores of this fungus upon the peach. Cunningham (15) states that in New Zealand teliospores are produced only on the plum. Cobb (13, pp. 232–233) stated that teliospores are usually absent on the peach but noted that, where the latter was in close juxtaposition to rusted plum trees, teliospores were found on both hosts. He suggested that there might be two different species or strains of rust involved in this case. Others have suggested the same possibility. McAlpine (27, p. 25) says: "It is remarkable that the teleutospores are comparatively rare on the apricot and peach and less so on the almond, but the plum seems to have some peculiarity in its constitution which stimulates the formation of the resting spores." Darnell-Smith (16) states that urediniospores and teliospores are found on peach. "As yet only two forms of spores are known for this species of *Puccinia*,—the uredo or summer spore, and the teleuto or winter spore. These spores are produced in varying proportions on different plants. On some hosts the uredospores greatly predominate over the teleutospores, while on other hosts the reverse is true. It appears probably that conditions of food, humidity, climate, and season all tend to vary these results. Both spore forms are produced on the under side of the leaves, and probably both may, under some conditions, serve as winter spores."

Aecial stage. Tranzschel, according to Arthur (3), successfully infected almond, *Amygdalus communis* L., blackthorn, *Prunus spinosa* L., and cherry plum, *P. divaricata* Ledeb., with aeciospores from *Anemone coronaria* L. and *A. ranunculoides* L. (*Aecidium punctatum,* described by Persoon in 1796.) Arthur (3, 4) found aecia on Anemone, Hepatica, and Thalictrum in the eastern United States and inoculated wild black cherry, *P. serotina* Ehrb., wild plum, *P. americana* Marsh, cultivated cherry, *P. cerasus* L., and peach, *P. persica* Sieb. and Zucc., with spores of *Aecidium hepaticum* Schw. from *Hepatica acutiloba,* obtaining infection only upon *P. serotina.* He repeated the inoculation the following year upon *P. serotina* and peach with the same result. He also inoculated peach with urediniospores from *P. serotina* "under seemingly most favorable conditions," but no infection resulted. Jackson (25, pp. 261–283) states that the aecial stage on Anemone has never been found in the West. Blasdale (10):

"Aecia not known from California but found on various species of *Hepatica, Anemone,* and *Thalictrum* in the eastern United States." Brooks (1), in England, successfully inoculated plum leaves with aeciospores from *Anemone coronaria.* Cunningham (15), in New Zealand, says "the aecidial host is an introduced species and is found in but a few localities; yet this rust is prevalent throughout New Zealand on stone fruits." He also states that "as viable uredospores are to be obtained nine months of the year it is probable that this is spread chiefly by these spores." Arthur (5) states: "The aecia are not always required for the propagation of the species, particularly in the warmer regions of its range, where the rust occurs in greatest severity, largely in the uredinial stage." Also: "On the twigs cankers are produced in which uredinia live from one year to the next and perpetuate the rust."

In the district in which this work was done no indication of an aecial stage of the fungus has been found; in fact, none of the probable hosts occur there, except possibly species of Thalictrum in the extensive river bottoms.

SUMMARY

A severe epiphytotic of a rust-fungus disease of peaches occurred in the principal canning-peach section of central California in 1926 and 1927. Serious economic loss was caused by disfiguration of the fruit, which ruined it for canning purposes, as well as by defoliation of the trees.

A description of the disease and the causative fungus is given.

The uredinial stage was the only form of the fungus observed.

Overwintering of the fungus was found to be dependent mainly on twig infections which originate in the fall, remain latent and invisible through the winter, and then develop as uredinial sori in early spring. The fungus also was carried over to a certain extent by overwintering of older sori on the previous season's twigs and by renewed production of urediniospores in spring from such sori.

Subsequent infection of leaves and fruit was found to be dependent upon the presence of sufficient inoculum and the occurrence of periods of several days of rainfall and high humidity.

For the germination of urediniospores a period of at least 3 hours in moisture-saturated atmosphere was found necessary.

The viability of urediniospores attached to living leaves was found limited to a period of about 6 weeks. On detached dried leaves the spores were somewhat shorter-lived.

Urediniospores of the peach-rust fungus germinated over a range of temperature between 8° and 38° C. The optimum temperature was found to lie between 13° and 26° C. The best growth of the germ tubes was observed at about 22° C.

Sulphur and sulphur compounds were found to be more toxic than copper to germinating urediniospores of the fungus. Mineral oil seemed to stimulate germination.

The identity of the fungus with relation to the prune-rust fungus *Tranzschelia punctata* (Pers.) Arth. (*Puccinia pruni-spinosae* Pers.) is discussed. In the present instance no spore form other than the uredinial was found on the peach and the fungus did not attack trees of other species of Prunus (prune, almond, apricot, cherry), even when they were growing immediately adjacent to badly affected peach trees. In the Santa Clara Valley, California, it was observed that the telial stage of the rust usually develops very abundantly on the leaves of prune trees in late fall, but the fungus was not found on adjacent peach trees.

No aecial form of the fungus was observed.

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THE INFLUENCE OF HYDROGEN-ION CONCENTRATION AND OF SODIUM BICARBONATE AND RELATED SUBSTANCES ON PENICILLIUM ITALICUM AND P. DIGITATUM[1]

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INTRODUCTION

Plant pathologists have in recent years become dissatisfied with their work in controlling diseases in the mere knowledge that a certain substance is effective in destroying a causative organism and have begun to inquire just how the results are brought about. The new viewpoint calls for a physiological investigation of the organism, particularly in the case of fungi.

The behavior of microorganisms in their relationships to acid and alkali is a phase of the physiology of fungi which has received much attention from investigators since the end of the last century. A complete review of the investigations of the earlier workers on the effect of acid and alkali on microorganisms is given by Webb (23), Johnson (8), and Myers (14). It was not until the measurement of active acidity as apart from titratable acidity and alkalinity was made use of by the plant pathologists that a more exact understanding of the action of the hydrogen and hydroxyl ions on microorganisms was possible; therefore, the results obtained previous to this period can be regarded only as indicative.

The two most important citrus fruit-destroying fungi in California are *Penicillium italicum* Wehmer (blue contact mold) and *P. digitatum* Saccardo (green mold). Dr. H. S. Fawcett, upon examination of five-hundred boxes of stored oranges in four packing houses in central California in 1927, found that of the decayed fruit 52 per cent was due to green mold, 32 per cent due to a mixture of blue and green molds, and 11 per cent due to blue mold, a total of 95 per cent. The rapid development of the citrus industry in California after 1900 resulted in a large increase in the quantity of fruit shipped, and investigations were commenced on the control of various decays that were responsible for considerable loss by the time the

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fruit had reached the markets, particularly those in the east. At that time the measures adopted were more in the nature of decay deterrents. Woodworth (28) mentioned refrigeration, ventilation for removing excess moisture, and wrapping the fruit to absorb moisture. For packing houses, sanitation was the main feature. Two angles of the cause of decay soon afterwards received attention: (1) the condition of the fruit which allowed infection and (2) the source of decay. Greater care in the handling of the fruit during picking, washing, and packing reduced decay considerably by reducing the number of wounds in the rind, which provide ideal places for natural inoculation. The treatment of the fruit itself was commenced after Smith (21) found that the washing tank was one of the main sources of infection of lemons by the brown-rot organism, *Pythiacystis citrophthora* Sm. and Sm. The addition of either Formalin, permanganate of potash, or copper sulphate to the water in the washing tank was advised. However, this treatment was not satisfactory for the control of blue and green mold on oranges, and, when experimentation with borax, then already a well-known preservative for articles of food, showed that by immersing the fruit in a five per cent solution almost complete control of green mold and some control of blue was obtained, it was not long before packing-house machinery was designed which would permit the commercial use of this process.

In 1927 Mr. A. H. Morgan, an orange grower in Rialto, California, found that the treatment of oranges with sodium bicarbonate gave as good control of the decay due to these molds as did borax or a mixture of borax and boric acid. Also, at about this time, the holders of the patent rights for the use of borax in the prevention of mold decay of citrus fruits, following a favorable court decision, started to enforce their rights to royalties for its use. Since the inventor of the sodium bicarbonate process had taken out a patent and given the free use of his process to the orange growers for ten years, a change was made in most packing houses from borax to bicarbonate when it was found that the latter was successful on a commercial scale. Although the use of sodium bicarbonate is more effective than borax in reducing blue contact mold, it is not so effective against green mold.

Here then is the problem: In just what manner is the prevention of decay effected by these substances, sodium bicarbonate in particular, and are the differences in reaction of two such closely related fungi to the treating substances such as to confirm the observations made in practicing decay prevention? Further, is the action of sodium bicarbonate specific or does it owe its action to some general physiological phenomenon?

MATERIAL AND TECHNIQUE

Since it is well known that strains of a certain fungal species will show differences in physiological behavior, it appeared desirable to conduct the

investigations with material from standard cultures. Hence, cultures were propagated from a single colony. The originals, *Penicillium italicum*, No. 1437, and *P. digitatum*, No. 1438, were obtained from the stock cultures of the Division of Plant Pathology, Citrus Experiment Station, University of California, Riverside. The blue contact mold organism is discussed as *Penicillium glaucum* Link in early papers, Smith (20), Powell (16), where its agency in the decay of citrus fruits is fully considered.

The determination of H-ion concentration was made by means of the quinhydrone electrode.

MEDIA

The first phase of the preliminary investigation was concerned with obtaining a satisfactory synthetic liquid medium on which the two fungi would grow equally well. Using 150 cc. Erlenmeyer flasks, a series was inoculated for each fungus for each of the following media: Czapek's, Duggar's, Richard's, Peptone, Pfeffer's, and Coupin's. The ten flasks per series were adjusted so as to have a hydrogen-ion range of from about pH 2.0 to pH 10.0. The adjustments were made with N/5 HCl and N/20 KOH by means of the method used by Karrer and Webb (9). Observations on growth were made daily. While *Penicillium italicum* grew excellently on Czapek's, Duggar's, Pfeffer's, and Coupin's solutions over a fairly wide range of pH, the behavior of *P. digitatum* was unsatisfactory on all.

Thom (22) states that *Penicillium digitatum* refused to grow on synthetic media containing nitrogen as sodium nitrate. To determine whether the nitrate ion was toxic to this fungus or whether it was merely unable to use it as a source of nitrogen, Duggar's solution was made up in four lots with nitrogen supplied as ammonium sulphate, ammonium nitrate, sodium nitrate, and sodium nitrate plus peptone. It was found that only 2 per cent germination and no growth were obtained with the sodium nitrate solution, as compared with 30 per cent germination and good growth with the other solutions. Considering both these results and those from preliminary culture work, it seems that this species of Penicillium cannot utilize the nitrate ion, while the ammonium ion and peptone are usable as a source of nitrogen with some difficulty.

While *Penicillium italicum* formed an excellent mat in about 8 days on the media just mentioned, it took *P. digitatum* twice as long to form a mat which could be used for comparative studies. Since the latter fungus is specific on citrus fruits and the former fairly cosmopolitan, it was thought that the influence of the various portions of the orange would give a clue to the differential nutrient requirements.

Twenty grams of cane sugar per liter were added to strong water extracts of the oil-bearing tissue (*flavedo*), the white of rind (*albedo*), the

TABLE 1.—*Effect of addition of orange juice and total orange extract to Modified Duggar's Solution on the growth of Penicillium digitatum. Time grown, 8 days*

Amount added per 50 cc. of medium	Initial pH	Orange juice		Orange extract	
		Final pH	Dry weight of mats, in gms. (Average of 3 mats)	Final pH	Dry weight of mats, in gms. (Average of 3 mats)
Checks (10 flasks)	6.7	6.4	No growth except a trace in two flasks		
2 drops	6.7	3.0	.132	3.4	.087
5 drops	6.7	3.2	.136	3.2	.153
10 drops	6.6	2.5	.198	2.7	.219
2 cc.	6.4	2.2	.371	2.6	.350
5 cc.	6.2	2.2	.398	2.3	.376

total rind, the pulp, and the total orange. Mycelial growth of both fungi was poor but sporulation very dense on the first two extracts, while on the remaining three both produced excellent mats and normal sporulation. Whether this marked stimulation in growth of *Penicillium digitatum* was due solely to the organic nitrogen supplied is doubted in view of the results shown in table 1. To determine in greater detail the influence of such substances in citrus fruits on the growth of these fungi on synthetic liquid media, an orange extract was produced by grinding up the whole orange, adding one liter of water to each kilogram of mash, autoclaving at 14 pounds pressure for 30 minutes, then straining through cheesecloth, and again autoclaving for 30 minutes. Orange juice was obtained from fresh fruits with a reamer and then autoclaved. From 2 drops to 10 cubic centimeters of these substances were added to flasks containing 50 cc. of sterilized Duggar's solution by means of pipettes. This work was done in a well-sprayed inoculating chamber. Eight days after inoculation the mats were taken out and the dry weights determined.

No growth was obtained in 8 of the 10 check flasks. The two remaining flasks showed some mat formation. They had received an extra heavy inoculation, tufts of sporulating mycelium having been introduced in addition to the spores as added to the other flasks. The addition of two drops of orange juice, having 1.6 per cent total solids which gave 18 per cent ash, allowed an average growth of 0.132 gm. dry weight. When the amount added was increased to 2 cc. and 5 cc. the increase in weight of mat formed was considerable and may be partly explained by the addition of an extra supply of nutrients. The addition of the ash of the juice failed to give the stimulation observed with the juice itself. No significant difference is

noted between the influence of the orange extract and the orange juice, while similar preparations from grapefruit and lemons in subsequent experiments gave similar results.

Williams (27) has shown that for normal growth baker's yeast was dependent on an adequate supply of a water-soluble vitamin in the medium. Willaman (26) found that a small amount of prune, peach, or apple juice and decoctions from other plant materials added to synthetic media on which *Sclerotinia cinerea* would make no or extremely weak growth allowed the fungus to grow at a normal rate. These decoctions and juices apparently supplied a substance which behaved as a vitamin.

Whether it can be postulated that *Penicillium digitatum* requires for its growth the presence of a vitamin found in citrus fruits, hence explaining its specificity, is problematical. Unfortunately, the continuation of this phase of the nutrition of this fungus lies outside of the scope of the present paper, but repeated experiments confirmed this influence of the orange extracts. As a result of this phase of the work a synthetic liquid medium of which the proportions of the principal nutrient constituents were known and which allowed approximately the same rate of development of excellent mats of both fungi was now available.

The writer has decided to name the synthetic medium used "Modified Duggar's Solution." The modifications include the use of ammonium sulphate since the nitrate ion has been shown to be unusable by *Penicillium digitatum,* the monohydrogen phosphate because its use results in a pH value of the solution nearer the neutral point, and a reduction in the amount of magnesium sulphate to lessen the amount of precipitate formed in alkaline solutions. The chemicals used were the usual C.P. salts, and these contain impurities in sufficient quantities to supply all the essential elements for fungal growth.

Modified Duggar's Solution:

$(NH_4)_2SO_4$ 10 gms.; K_2HPO_4 5 gms.; $MgSO_4 \cdot 7H_2O$ 1 gm.; $FeSO_4$ trace; cane sugar 25 gms.; H_2O 1 liter.

From 2 to 5 per cent orange extract was added to the medium at time of making, the percentage depending on the amount available.

Orange-glucose-potato agar was found to give a more vigorous growth for both fungi, with plentiful sporulation, and was used throughout the work. Five per cent of sterilized orange extract was added when filling the test tubes for slants, for it was found that if the orange juice was autoclaved with the agar the latter would not harden. This process was performed in the inoculating chamber, the test tubes and plugs having been sterilized before filling, and a re-autoclaving after filling was not necessary.

Cultures. The liquid medium was made up in large enough quantities for each complete experiment. Each flask contained 50 cc. of the medium,

Erlenmeyer flasks of 150 cc. capacity being used throughout. Spores from slants were transferred to the culture flasks by means of a chromel wire loop. It was found that spores from cultures up to three months old did not vary in percentage of germination, and as long as an even distribution of spores over the surface of the medium was obtained the final mat weight was not influenced by the inoculation process.

The age of the culture of a fungus at which the mat attains maximum weight varies mainly with the medium and the temperature at which grown. The influence of temperature on growth was minimized by setting up a complete experiment at once, the flasks being kept on trays at room temperature. Fawcett and Barger (6) showed that the two fungi concerned here were generally affected similarly by different temperatures. To determine the approximate length of time cultures should be grown to obtain best results before autolysis sets in, a series of flasks for *Penicillium italicum* and *P. digitatum* was placed in the incubator at 21.5° C. Four flasks were removed daily, the mats washed in water, dried in the oven for 24 hours at 95° to 98° C. and weighed. The growth curves for the two fungi are almost similar, with *P. italicum* reaching maximum weight in 6 days and *P. digitatum* in 7 days; autolysis is slow up to 12 days (Fig. 1). Since conditions

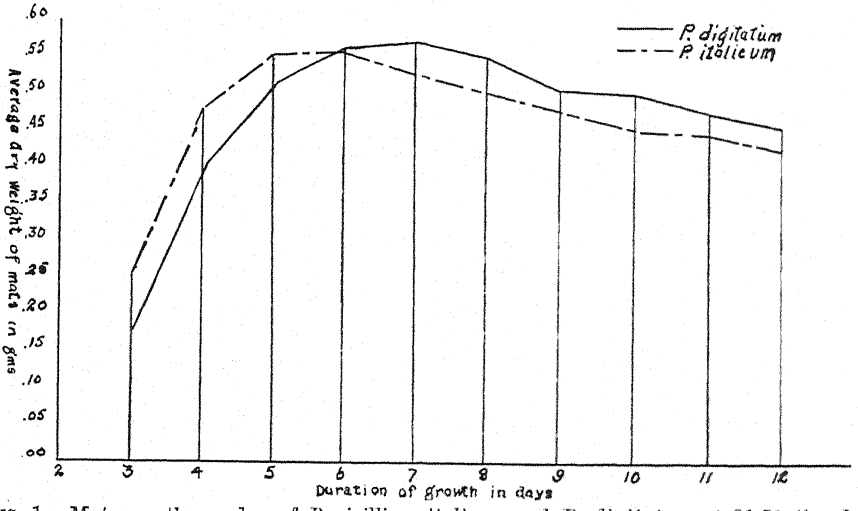


FIG. 1.—Mat growth per day of *Penicillium italicum* and *P. digitatum* at 21.5° C. In modified Duggar's solution + 10 per cent orange extract + 1 per cent potassium citrate (50 ml. per flask).

here were almost optimum for mat growth, it was decided to grow all mats for 8 days in future experiments to allow for lagging in growth due to any slightly unfavorable conditions. The final hydrogen-ion concentrations of media were always determined.

Germination. For the germination experiments Van Tieghem cells were sealed with paraffin to glass slides, two cells to each slide. The bottom of the cell was covered with distilled water and a touch of vaseline put at two opposite spots on the upper edge of the ring, upon which the cover glass rested. With germination experiments in different media, suspensions of spores, obtained from slant cultures about one month old, were made. A drop of each suspension was then placed on a cover glass and inverted over its respective cell. This was done in duplicate for all determinations. When germination had proceeded the desired length of time, the cover glasses were raised and a few drops of chloroform introduced into the bottom of the cells. This was done to stop germination and growth during periods of observation and measurement.

Where the spores were subjected to different treatments by bringing them in contact with various alkali solutions, a slight modification of the technique of Henderson Smith (7) was used. Spores were introduced into 15 cc. of the treating substance in a test tube, well shaken, and 1 cc. of the suspension run into 10 cc. of distilled water in a centrifuge tube at the exact completion of the time of treatment, the time being noted on a stop watch. This was then centrifuged for 10 minutes, the water poured off and a few cc. of Modified Duggar's Solution plus orange extract squirted into the tube, making a new suspension from which hanging-drop cultures were made.

Germinated spores which were to be subjected to different treatments were obtained by dusting a very thin film of spores on Modified Duggar's Solution (in further references to Modified Duggar's Solution it will be understood that 5 per cent orange extract has been added unless otherwise stated), just covering the bottom of a large flat specimen dish. When examination showed that germination was well under way, the germ tubes not being too long, the medium was poured out and a suspension made. This suspension was then added to the treating substances, the amount being determined by the heaviness of the suspension, and the process was continued as described above. Observations on the amount of germination were made on both cells on each slide, three fields in each cell generally being counted. Careful counts were made in all cases where the germination was less than 20 per cent, for it was in this range that results that would count were looked for.

HYDROGEN-ION-CONCENTRATION STUDIES

In obtaining the suitable medium already mentioned, it was noted that the pH of the media after supporting growth showed great variation both within each series and between different media, but that the results produced by the two fungi were somewhat similar. The change in pH from

the initial value seemed to have a relation to the amount of growth. Klotz (10) showed that *Aspergillus niger* began autolyzing after 3 days, the disappearance of the carbohydrates from the culture medium being synchronous with the beginning of autolysis. By growing *Penicillium italicum* on Czapek's solution, the writer found that this fungus autolyzed extremely rapidly after the ninth day where the initial hydrogen-ion concentration of the medium was pH 4.0. The medium showed a marked change in pH towards the alkaline side only after autolysis had set in. That *P. italicum*

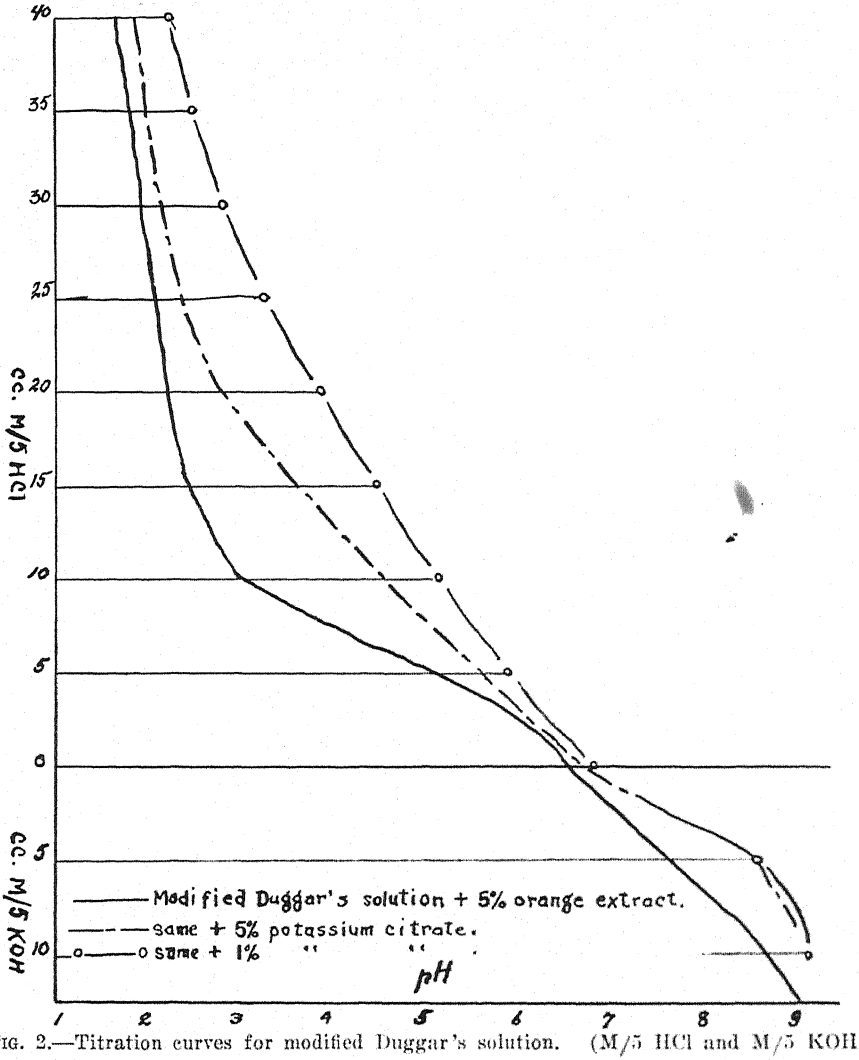


FIG. 2.—Titration curves for modified Duggar's solution. (M/5 HCl and M/5 KOH titrated into 50 ml. medium. Determinations by quinhydrone electrode.)

produces oxalic acid when grown on media containing sucrose has been shown by Currie and Thom (5), the production being considerable when calcium carbonate is present to remove the acid in combination when formed. Since the two fungi here studied changed the reaction of the medium similarly, it is accepted that *P. digitatum* also has oxalic acid as one of the main products of metabolism.

The buffer systems of the medium will largely determine the final pH if the amount of growth is the same at different initial pH values. This change in reaction of the medium invalidates experiments to find the optimum hydrogen-ion concentration for growth where only the initial values are known; the medium has to be kept at constant pH for true results. To be able to interpret comparatively the changes in reactions of the media due to the fungus growth, electrometric titrations of Modified Duggar's Solution with and without potassium citrate were made. The titration curves are shown in figure 2. The addition of citrate allows fungi to be grown over a wider range of hydrogen-ion concentrations of the media without the differential change in the reaction influencing final results. The direct and indirect influence of the citrate on the growth of the fungi was tested out in a comprehensive experiment in which the concentrations of citrate and sucrose in the medium were varied. The results are shown graphically in figure 3. It is seen that neither fungus can utilize the citrate as a source of carbon for metabolism when no sucrose is present. Camp (3) found that citrate mixtures adjusted to a favorable pH value proved to be efficient as a supplementary carbon source when used with small quantities of dextrose for all the fungi he worked with except *Penicillium digitatum* and *Phomopsis citri*. Sakamura (18) holds that the favorable influence on the growth of *Aspergillus niger* by the addition of oxalate, citrate, or phosphate of potassium is due to its helping to maintain a favorable pH in the media. The increase in the amount of sucrose when no citrate was present resulted in almost proportional increase in mat growth in the results recorded here, but the mat growth in the medium containing 20 gms. sucrose and 10 gms. citrate is greater than that in which 30 gms. sucrose is present. Whether this extra growth is due to the ability of the fungus to utilize the citrate or whether it was because the medium maintained a hydrogen-ion concentration most favorable for growth cannot be stated, but the writer thinks it is mainly because a favorable reaction is maintained in the medium.

The growth of fungi in a medium of constant hydrogen-ion concentration was attempted by Sideris (19), but the nearest approach to such conditions has been attained by Marloth (13) with his apparatus which makes possible the repeated renewal of the medium under the fungus mat. An improvement on that apparatus is offered in figure 4. The tube G has been

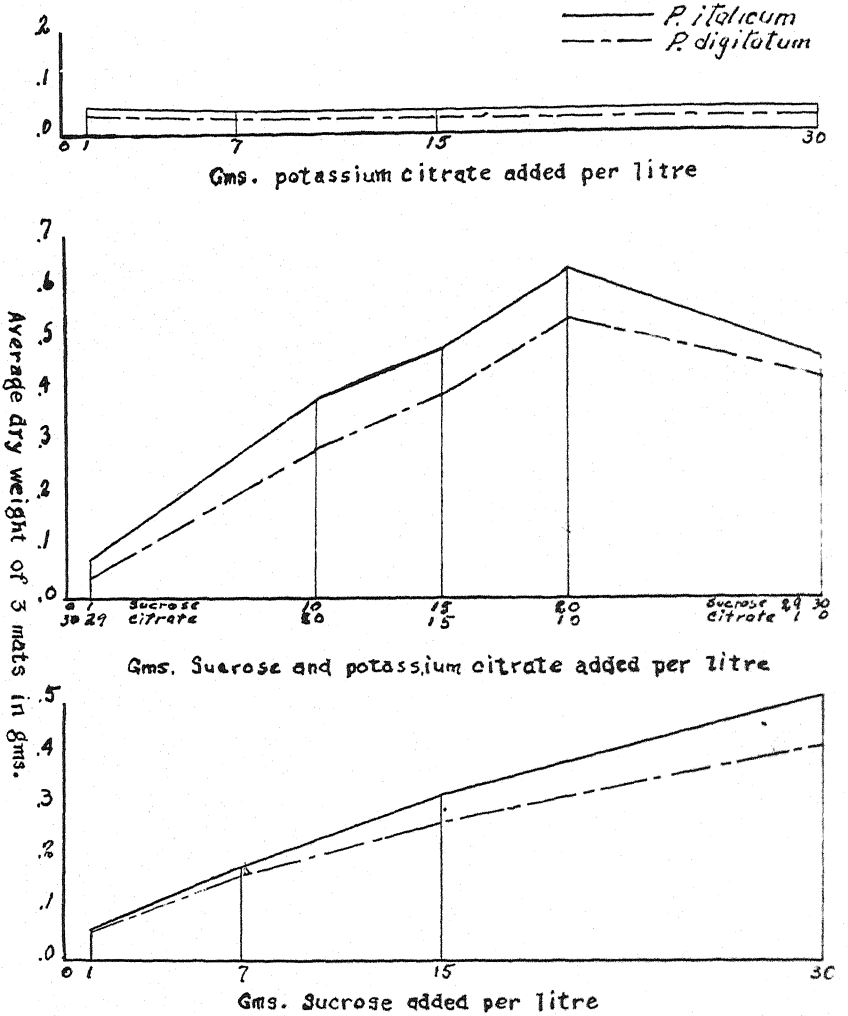


FIG. 3.—Effect on growth of varying the proportions of sucrose and citrate in the medium. (Modified Duggar's solution + 2 per cent orange extract.)

extended to the bottom of the flask to prevent the new supply of medium lodging on top of the mat. Furthermore, round-side flasks are more satisfactory than Erlenmeyer flasks.

Using the improved apparatus with 70 cc. Modified Duggar's Solution in each flask, cultures were grown as follows:

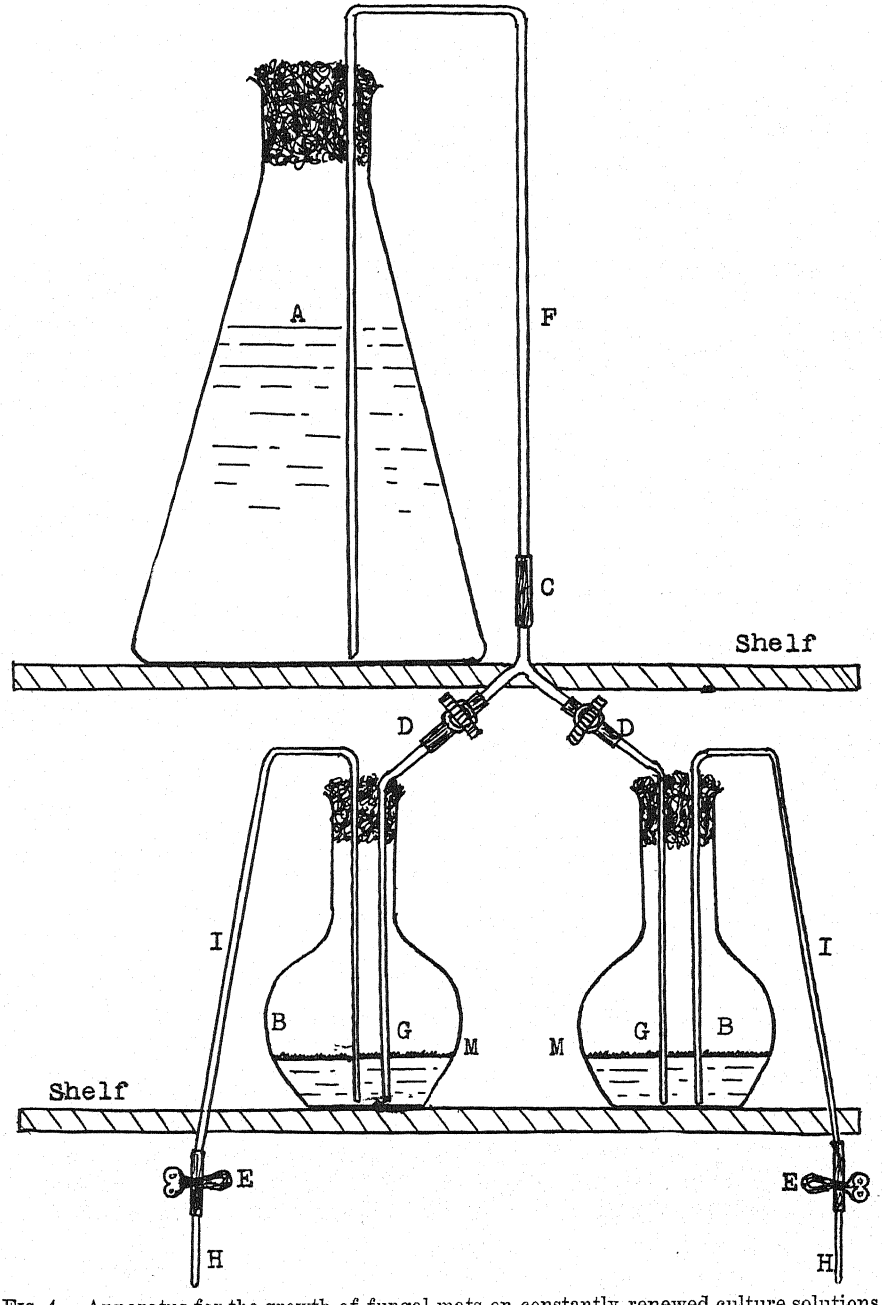


FIG. 4.—Apparatus for the growth of fungal mats on constantly renewed culture solutions.

No citrate					
P. italicum, 8 days					
Initial pH	2.05	2.5	3.9	4.6	6.0
Av. dry wt. of 2 mats, in gms.	.139	1.058	1.265	1.663	1.383
P. digitatum, 9½ days					
Initial pH	2.3	2.7	4.9	5.9	6.5
Av. dry wt. of 2 mats, in gms.	.032	.822	.818	.667	.376
1 per cent potassium citrate					
P. italicum, 8 days					
Initial pH	2.5	3.15	5.6	6.05	6.8
Av. dry wt. of 2 mats, in gms.	1.160	2.947	2.357	2.049	1.631
P. digitatum, 8 days					
Initial pH	2.5	3.0	3.7	5.0	6.75
Av. dry wt. of 2 mats, in gms.	.137	1.898	1.935	1.699	.394

The "no citrate" and the "citrate" are not comparable, due to the former having been grown during cold weather and the latter during very warm weather. Further, in the case of the no citrate series, the daily drop in the pH of the medium was relatively large, from pH 5.6 to 4.1 for *Penicillium italicum* and from pH 5.8 to 4.3 for *P. digitatum* after the mats were well formed. With the citrate series the daily change in reaction was smaller, the greatest being from pH 5.6 to 4.8 for *P. italicum* and from pH 6.7 to 5.9 for *P. digitatum*. These marked changes in reaction usually occurred with media having an initial pH above 5.0, whereas the change in media with pH 2.5 was very slight. Table 2 shows the daily drop in pH value due to the growth of *P. digitatum* on medium plus citrate. Check flasks grown on 70 cc. of medium for 8 days gave a final pH of 2.1 for *P. italicum* and 2.45 for *P. digitatum* when no citrate was present as a buffer. No cultures have been found to have a lower final pH than 1.9 and 2.2, respectively, for these two fungi. This final hydrogen-ion concentration of the media depends somewhat on the initial pH, for when no citrate was present as a buffer the reduction was from 2.7 to 2.0, 4.4 to 2.5, and 6.4 to 2.6 for *P. italicum* and from 2.7 to 2.2, 4.4 to 2.4, and 6.4 to 2.5 for *P. digitatum* (Table 3).

The limits of the hydrogen-ion concentration of the media which would allow growth of the fungi under investigation were not exactly determined, but it is seen that they have a fairly wide optimum range. Numerous cultures have indicated that *Penicillium italicum* will not form a mat if the initial pH of the medium is below 2.1 or above 7.9, and *P. digitatum* not below 2.4 or above 7.8. Particularly in the case of the latter fungus the mats are very unsatisfactory above the neutral point, being generally submerged. However, a fair amount of mycelium will develop within the liquid medium up to pH 8.5.

TABLE 2.—*Growth of Penicillium digitatum in flasks. Medium renewed daily, with 70 cc. Modified Duggar's Sol. + 5 per cent orange extract + 1 per cent potassium citrate; also daily increase of hydrogen-ion concentration of medium*

Initial pH of medium	Daily pH of renewed solutions in flasks							Final pH in reservoir	Dry weight of mats, in gms.	
	Days								Duplicate flasks	Average
	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth			
6.75	6.45	6.25	6.15	6.1	6.0	5.95	5.9	6.7	.376 .412	.394
5.0	4.9	4.7	4.7	4.65	4.7	4.75	4.8	5.25	1.722 1.676	1.699
3.7	3.65	3.5	3.4	3.5	3.5	3.55	3.55	3.9	1.945 1.722	1.985
3.45	3.2	3.1	3.0	3.0	3.0	3.15	3.2	3.4	1.907 1.788	1.848
2.95	2.9	2.8	2.65	2.6	2.6	2.55	2.55	3.0	1.836 1.960	1.898
2.5	2.5	2.5	2.45	2.45	2.4	2.4	2.4	2.5	.152 .122	.137

TABLE 3.—*Mat growth per day in flasks containing Modified Duggar's Solution + 5 per cent orange extract*

Days of growth	Initial pH 2.7			Initial pH 4.4			Initial pH 6.4		
	Final pH	Av. dry wt. of 2 mats, in gms.	Change in dry wt.	Final pH	Av. dry wt. of 2 mats, in gms.	Change in dry wt.	Final pH	Av. dry wt. of 2 mats, in gms.	Change in dry wt.
Penicillium italicum									
3	2.65	.....	.....	3.1	.158	+.158	4.0	.088	+.088
4	2.4	.133	+.133	2.7	.272	+.114	3.4	.193	+.105
5	2.25	.268	+.135	2.55	.362	+.090	2.8	.337	+.144
6	2.15	.369	+.101	2.5	.403	+.051	2.65	.439	+.102
7	2.05	.423	+.054	2.5	.412	+.009	2.6	.458	+.021
8	2.0	.489	+.066	2.5	.429	+.017	2.6	.461	+.003
10	2.0	.523	+.034	2.65	.408	−.021	2.8	.439	−.022
12	2.05	.476	−.047	2.8	.376	−.032	2.85	.430	−.009
Penicillium digitatum									
3	2.65	.....	.....	2.7	.252	+.252	4.45	.097	+.097
4	2.5	.038	+.038	2.5	.406	+.154	3.3	.211	+.114
5	2.4	.099	+.061	2.4	.472	+.066	2.8	.348	+.137
6	2.35	.155	+.056	2.45	.493	+.021	2.55	.475	+.127
7	2.25	.228	+.073	2.4	.488	−.005	2.55	.472	−.003
8	2.25	.274	+.046	2.45	.485	−.003	2.6	.446	−.026
10	2.2	.359	+.085	2.55	.423	−.062	2.65	.430	−.016
12	2.2	.360	+.001	2.6	.410	−.013	2.9	.414	−.016

The morphological nature of the mats of both of these fungi was directly influenced by reaction of the media, those grown on media of pH 2.6 and below being brittle and wrinkled with no or only slight sporulation, while they were slimy with excellent sporulation on media of pH 6.0 or above.

Two series of cultures of 64 flasks each were grown, one with citrate and the other with no citrate in the medium, three initial pH values of 2.7, 4.4, and 6.4 being used for each series. Two flasks were removed daily, the final pH determined, and the mats dried and weighed. The results as shown in table 3 bring out two points already noted; namely, that *Penicillium digitatum* grows more rapidly than *P. italicum* and that the addition of citrate to the medium results in better growth for both fungi. The three initial pH values of the media were chosen with 4.4 as the optimum for growth and 2.7 and 6.4 as being near the acid and alkaline limits. The wider range of tolerance for hydrogen-ion concentration is shown by *P. italicum*. Further, this fungus reduced the pH value of the medium to 2.0 and *P. digitatum* to 2.2 in 8 days, and these figures may be taken as the lower limit for growth, the latter fungus not having so low a limit as *P. italicum*.

Whether the maximum hydrogen-ion concentration of a medium in which growth will start or which will allow growth to continue should be regarded as the lower limit is debatable, but nevertheless it remains that *Penicillium italicum* is more tolerant towards acid for growth than *P. digitatum*. This order of tolerance remains true also on the alkaline side, for abnormal and restricted growth occurs with *P. digitatum* at a pH value where *P. italicum* forms almost normal mats.

Germination. The germination of spores of fungi in relation to the hydrogen-ion concentration of the media has received excellent study by Webb (23, 24). In his first paper he records that the maximum germination for most fungi studied was between pH 2.8 and 3.1. The highest reaction which allowed germination, although slight, was pH 10.0 at 27° C., *Penicillium cyclopium* being the fungus. The second paper records the limits and optimum range of germination for certain fungi on different media at various temperatures. He found that *P. italicum* had the following ranges: Mannite: Ortho-phosphoric acid 2.0 to 7.7, Czapek 1.8 to 8.2, peptone 1.6 to 7.2, beet decoction 2.4 to 8.9. The optimum range was pH 3.0 to 4.0 for all media.

Webb summarizes as follows: "On comparing equal concentrations of H and OH ions, the OH ions appear to be relatively more toxic to spores studied than H ions. The toxicity of H ions is fairly independent of the other conditions studied, while that of OH ions tends to be more or less variable or antagonizable, according to the composition of the medium."

TABLE 4.—*Effect of pH of medium on germination of spores in Van Tieghem cells. Modified Duggar's Sol. + 5 per cent orange extract. No citrate*

pH	Percentage of germination			
	Penicillium italicum		*Penicillium digitatum*	
	24 hrs.	60 hrs.	24 hrs.	60 hrs.
1.5	0	0	0	0
1.95	0	15	0	0
2.4	25	75	10	40
3.0	80	95	70	80
3.7	90	E	80	90
5.3	90	E	80	E
6.1	90	E	70	E
6.5	90	E	70	E
7.05	60	E	50	E
7.1	50	E	60	E
7.8	40	E	60	E
8.2	40	E	50	E
8.4	60	E	70	E
9.0	50	E	40	E

E = Hanging drop overgrown, thus unable to count spores.

TABLE 5.—*Effect of pH of medium on the germination of mature spores in Van Tieghem cells. Modified Duggar's Sol. + 5 per cent orange extract. No citrate. Set up in duplicate. Temperature 24°–30° C.*

pH of hanging drop	Percentage of germination in 19 hours			
	Penicillium italicum		*Penicillium digitatum*	
6.55	100		45	
7.85	98		40	
8.1	99		32	
8.2	98		45	50
8.4	98		42	50
8.6	97		50	
8.9	96		31	20
9.2	98		52	60
9.4	62	85	37	57

The wider range of tolerance for H ions exhibited by *Penicillium italicum* in comparison with *P. digitatum* for growth finds a parallel in the germination experiments recorded in tables 4 and 5, especially on the acid side. Of the media used, Modified Duggar's Solution allowed maximum germination. A series of cells was set up with the medium used for the

hanging drops adjusted to 14 different pH values. The resulting percentage of germination after 24 and 60 hours is presented in table 4. Here the lower limit for *P. italicum* is 1.95 and 2.4 for *P. digitatum*. A small subsequent similar experiment gave 3 per cent germination at pH 1.85 and 2 per cent at pH 2.75, respectively, for the two fungi, and these values are presented as the lower limits of germination on this medium. The amounts of germination recorded on the alkaline side are subject to criticism, for, while the initial pH of the hanging drop was as is given in the table, reference to figure 2, which gives the titration curve for the medium, will show how weak the buffer system is in that range. This explains why media adjusted to pH values on the alkaline side change towards the neutral point, even when merely standing in cotton-stoppered flasks, independent of the change in reaction of the hanging drop due to the first slight growth of the spores. A repeat experiment is recorded in table 5. In this the period for germination was shortened and the cover glasses were sealed to the rings to exclude the entrance of a constant supply of carbon dioxide from the air. In addition, the pH was determined for flasks of the adjusted media before and after the experiment and the lower value given as the pH of the hanging drop. A retardation of germination is noted above pH 9.2 for *P. italicum,* but the behavior of *P. digitatum* was very variable. As it was not possible to adjust Modified Duggar's Solution to pH values higher than 9.6 without the addition of so much alkali as to radically alter the composition of the medium, the upper limit of germination for these fungi on this medium has not been determined, although it was found that *P. italicum* gave only 20 per cent germination and *P. digitatum* 10 per cent at pH 9.7. Using Sörensen's glycocoll buffer mixture, as given in Clark (4), no germination for either fungus was obtained above pH 9.7. No germination of the spores of either of the blue or green molds was obtained in 2.6 and 10 per cent solutions of sodium and potassium carbonate and bicarbonate and 4 per cent sodium borate in water to which 2 per cent sucrose had been added. The pH values of the bicarbonate solutions were 8.3 to 8.6, of the carbonates, 11 to 11.4, and of the borate, 9.4. An interesting observation in the preliminaries to this experiment was that the percentage of germination of both fungi was twice as great in sucrose dissolved in tap water as in sucrose in distilled water and much less when dextrose was used instead of sucrose, the highest percentage of germination in any of these media being only 40 per cent. A further determination on the lower limit of germination was made, this time with KCl–HCl buffer solution plus 2 per cent dextrose. Again it was noted that *P. italicum* has a lower limit, pH 2.0 as against 2.2 for *P. digitatum,* the latter being relatively inhibited below 2.4.

INFLUENCE OF THE SODIUM ION

In studying the toxic properties of a chemical substance it is desirable to know the effect of the individual ions as well as that of the combined compounds on the microorganisms. With mercury salts it is known that their toxic properties lie in the mercury ion, as shown by Madsen and Nyman (12). Working with *Penicillium glaucum* (probably *P. italicum*) Boeseken and Waterman (2) concluded that the cause of the toxic action of certain metallic salts, as well as boric acid, is not purely physical, but may be primarily chemical; the most active elements form complex compounds within the organism. With sodium bicarbonate it is not even known whether the toxic property is an indirect or a direct one, that is, whether it lies in the whole or part of the compound or in the reaction caused by its dissolution in water. Levine, Buchanan, and Toulouse (11) showed that the addition of NaCl, Na_2CO_3, or Na_3PO_4 to NaOH markedly decreased the killing times for bacteria, suggesting that it is the undissociated NaOH which, penetrating the cell, causes death. That this is controversial is shown by Meyers (14) who states "that the disinfectant action of an alkali is one largely due to the concentration of free hydroxyl ions in solution has been generally accepted." He offers a review of the work of other investigators, most of which pertained to bacteria, and the alkalies used were strongly dissociating ones, such as hydroxides and carbonates. With sodium and potassium bicarbonate the reaction of a 6 per cent solution is only pH 8.5. Since it has been shown earlier that spores of *P. italicum* and *P. digitatum* would germinate in media of pH 9.7, the disinfectant property of sodium bicarbonate cannot wholly be explained by the hydroxyl-ion concentration of the solution.

To test the influence of the sodium ion on the growth and germination of the Penicillium species here under investigation, sodium citrate, potassium citrate, sodium chloride, and potassium chloride were added to Modified Duggar's Solution in varying concentrations, calculated in parts per million of sodium or potassium ions. Two series of cultures were grown with each salt, and the growth results are plotted in figures 5 and 6. The data were obtained from 150 cultures in which the cation concentrations of sodium and potassium citrate were from 2,500 to 20,000 p.p.m. Three flasks were used for each value. It was found that potassium citrate up to a concentration of 35,000 p.p.m. of potassium did not inhibit growth and germination of *P. italicum* and that *P. digitatum* was only slightly inhibited relative to the effects of the sodium salt. (References to "inhibition of growth and germination" mean fairly marked inhibition.) Sodium citrate has a marked inhibitory influence on the germination of *P. italicum* at 20,000 p.p.m. and on *P. digitatum* at 15,000 p.p.m. It inhibited the

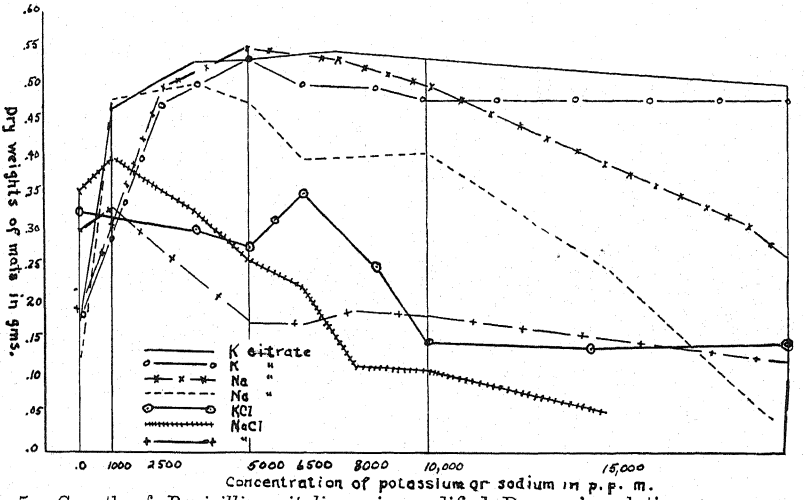


FIG. 5.—Growth of *Penicillium italicum* in modified Duggar's solution + orange extract + varying concentrations of potassium and sodium.

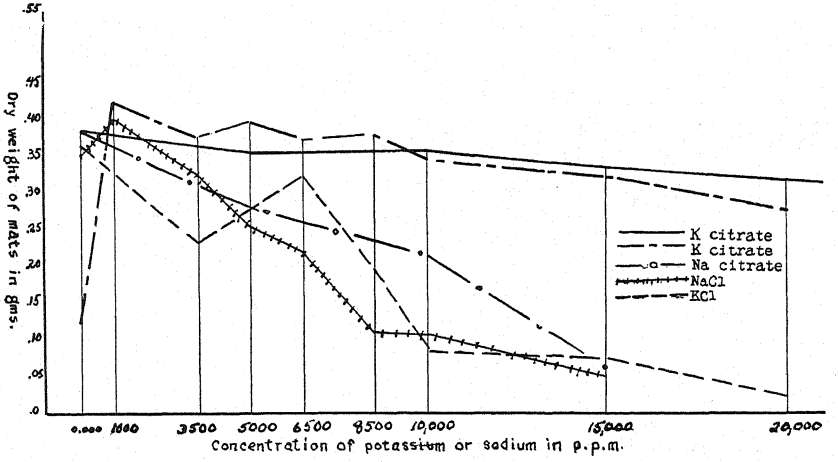


FIG. 6.—Growth of *Penicillium digitatum* in modified Duggar's solution + orange extract + varying concentrations of potassium and sodium.

growth of the latter fungus at 10,000 as compared with 20,000 p.p.m. for the former. Sodium and potassium chloride were similar in their inhibitory effects on the growth of both fungi, the dry weights of mats formed in the presence of 10,000 p.p.m. of sodium or potassium being negligible. The indications are that with these two salts it is the chlorine ion which causes inhibition of growth, especially as the potassium salt does not inhibit germination of *P. italicum* at all up to 20,000 and *P. digitatum* only above 15,000 p.p.m. Unfortunately, the amount of dissociated anions and

cations in the solutions used by the present method could not be calculated, so that the comparison of the toxic influence of the chloride and citrate salts based on the total p.p.m. of cations added is not quite justifiable. Throughout all of these series a favorable initial pH of the media was maintained and the final pH values in no case were beyond the usual limits. That the factor of the osmotic pressure of the solutions had no obvious influence on the results obtained is shown by the normal germination and growth when the medium contained 50 per cent sucrose with an osmotic pressure of 38 atmospheres as compared with 17.3 atmospheres for the solution containing 35,000 p.p.m. potassium as citrate.

In interpreting the recorded results the difference between the sodium and potassium ions seems to warrant the conclusion that the potassium ion up to the high concentrations used exerts no inhibitory action, while the sodium ion, whether added as citrate or chloride, is undoubtedly toxic to both fungi at the higher concentrations but to a greater extent to *Penicillium digitatum*.

EFFECT OF BICARBONATES AND CARBONATES ON SPORES

The critical pH values of the medium for growth of *Penicillium italicum* were found by Johnson (8) to be 1.9 to 2.2 and 9.1 to 9.3 on Czapek's medium, and the toxicity of salts, molecule for molecule, as follows: $KCl < NaCl < Na_2CO_3 < K_2CO_3$. Since the two latter salts give pH values of 10+ when in solution in the concentrations used, the major part of their toxicity is undoubtedly due to the high hydroxyl-ion concentration and to the bicarbonate ion, as will be shown later, when the pH of the medium is 8.2 or slightly higher after the addition of the salts. Pratt (17) shows that 5.6 per cent potassium bicarbonate in Richard's solution, giving a pH value of 8.4, allowed no germination of *Penicillium* sp. spores.

The percentage of the solution of sodium bicarbonate in which the citrus fruits are immersed for treatment is from 3 to 4 per cent, this having been found to be the most suitable concentration for use in commercial packing houses. While it has been found that better control is obtained with a 6 per cent solution it is not practical to use due to the heavy deposits of salt forming on the machinery which conveys the fruit. A 1 per cent solution of sodium carbonate causes injury to the fruit, and this concentration is not sufficient to give such good prevention of decay as the 3 per cent solution. The process used in packing houses is that after being washed in a soap solution with brushes the fruit is immersed in the bicarbonate solution heated to 100° F. for from 4 to 6 minutes. As the fruit comes out of the last tank a thin spray of water washes off the excess treating solution, after which it is dried by means of warm air and then wrapped. Barger (1) treated large quantities of experimentally injured, inoculated, and orchard-

run fruit in baths of sodium borate and sodium bicarbonate to which mixed spores of both fungi had been added. His results of 4 minutes soaking in solutions at 100° F. include the following amounts of decay after 6 weeks.

		Injured Fruit		
Solution:	Water	4½% sodium borate	3% sodium bicarbonate	5% sodium bicarbonate
Percentage of decay:	86.0	69.3	35.3	32.0
		Noninjured Fruit		
Solution:	Water	4–5% sodium borate	4–6% sodium bicarbonate	
Percentage of decay:	28.3	18.2	16.7	

Effect on nongerminated spores. Naturally one of the first questions to arise is whether the spores themselves have been killed or whether the fruit has been conditioned in some way so as to render the start of decay difficult. The effect on the germination of the spores of *Penicillium italicum* and *P. digitatum* of various concentrations of sodium and potassium carbonate and bicarbonate is given in tables 6 and 7, the former re-

TABLE 6.—*Effect of treatment of nongerminated young, mature spores. Temperature 25°–30° C.*

Treatment	Time, in min.	Per cent germination in 24 hours	
		Penicillium italicum	*Penicillium digitatum*
Check	.....	100	80
4% $Na_2B_4O_7 \cdot 10H_2O$	5	86	40
2% $NaHCO_3$	2	97	55
	5	91	50
6% $NaHCO_3$	2	72	15
	5	63	11
2% Na_2CO_3	2	85	90
	5	80	80
6% Na_2CO_3	2	78	50
	5	75	15
2% $KHCO_3$	2	95	90
	5	80	60
6% $KHCO_3$	2	80	60
	5	65	50
2% K_2CO_3	2	98	35
	5	80	25
6% K_2CO_3	2	95	25
	5	65	18

TABLE 7.—*Effect of treatment on nongerminated spores 6 days old. Temperature 20°–25° C.*

Treatment	Time, in min.	Per cent germination in 24 hours	
		Penicillium italicum	*Penicillium digitatum*
Check	.....	95	50
4% $Na_2B_4O_7 \cdot 10H_2O$	10	50	45
10% $NaHCO_3$	2	95	45
	5	90	40
	10	85	35
	25	25	20
	45	20	20
10% Na_2CO_3	2	90	40
	5	80	30
6% $KHCO_3$	2	95	45
	5	90	40
15% $KHCO_3$	10	85	40
	25	70	15
25% $KHCO_3$	10	25	10
	25	10	5
4: 1000 $HgCl_2$	5	0	0

cording results with young mature spores and the latter with spores 60 days old. The success of the experiments depends on the wetting of the spores so as to allow the treating substances to act on the spore wall or the protoplasm within. That this was accomplished is shown by the fact that the mercuric chloride-treated spores gave no germination, showing complete wetting. Since this was an aqueous solution of higher surface tension than the alkali solutions, it may be presumed that the latter completely wetted the spores. Following the two-minute treatment of all concentrations of the bicarbonates and carbonates the reduction in germination of the young mature spores of *P. italicum* is not great enough to be really significant, while with those of *P. digitatum* it is marked. However, a 4 per cent solution of sodium borate is more effective against the latter fungus than the former, this being the case in commercial treatments. Certain facts regarding both fungi are clearly indicated. The resistance of 60-day-old spores against the treating substances as compared with 14-day-old spores is quite marked for *P. digitatum*. The increase in concentration of the treating substances increases the extent of spore mortality. This is without any significant increase in pH value of the solutions, as shown by the values given in table 8. Increase in time of treatment increases the number of

TABLE 8.—*Effect of treating germinated spores. Temperature 22°–29° C. Percentage germinated before treating, Penicillium italicum 70, Penicillium digitatum 40*

Treatment	pH	Time, in min.	Per cent germination in 22 hours					
			Penicillium italicum			*Penicillium digitatum*		
			Total	Growth	Killed	Total	Growth	Killed
Check	7.0	.....	98	E	.....	98	E	.....
4% $Na_2B_4O_7 \cdot 10H_2O$		5	90	G	20	45	F	40
2% $NaHCO_3$	8.3	2	95	E	3	85	E	1
		5	95	G	10	85	E	3
6% $NaHCO_3$	8.4	2	90	F	10	75	E	5
		5	85	F	25	80	V.G.	10
		10	75	P	50	80	V.G.	30
10% $NaHCO_3$	8.5	2	90	G	15	55	F	30
		5	80	F	40	50	F	35
		10	75	P	60	50	P	35
2% Na_2CO_3	10+	2	85	G	15	60	G	15
		5	80	F	25	55	F	20
6% Na_2CO_3	10+	2	80	F	20	50	F	20
		5	70	P	40	45	P	30
10% Na_2CO_3	10+	2	80	F	15	45	P	40
		5	75	V.P.	50	45	V.P.	40
		10	70	V.P.	65	40	None	40
2% $KHCO_3$	8.4	2	95	G	2	80	V.G.	3
		5	90	G	10	75	G	8
6% $KHCO_3$	8.5	2	90	G	10	60	F	15
		5	80	V.P.	50	45	P	35
2% K_2CO_3	10+	2	80	P	10	50	F	15
		5	75	P	25	45	F	20
6% K_2CO_3	10+	2	75	P	15	50	P	25
		5	70	V.P.	50	40	None	40
4:1000 $HgCl_2$	3.0	5	70	None	70	40	None	40

E = Excellent; G = Good; F = Fair; P = Poor; V.P. = Germ tubes not more than 20 μ in length.

spores killed. The sodium salts are slightly stronger disinfecting agents than the potassium salts. The same percentage of carbonate is more effective than the bicarbonate in killing the spores. Nevertheless, the reduction in germination, with the exception of 25 per cent potassium bicarbonate solution for 25 minutes, is not sufficient to explain the degree of prevention of decay obtained in the commercial process. It is true that after the spores have been in the treating tank of sodium bicarbonate for several

hours all will have been killed, but there are sufficient spores in the atmosphere of the packing houses settling on the fruit as it passes along the conveyers after treatment to nullify the results of treatment unless some other factor or factors were involved. The writer believes that he has found this factor to be the thin film of sodium bicarbonate left on the rind of the fruit after drying.

Effect on germinated spores. In the experiments with germinated spores, as mentioned previously, a dusted film of spores was allowed to germinate on liquid medium. Due to the great difference in the rate of germination in such a film, the suspension had to be made when only 70 per cent of the spores of *Penicillium italicum* and 40 per cent of *P. digitatum* had germinated, for otherwise the germ tubes which appeared first would have increased to such a length as to render the making of the suspension and subsequent treatment impossible without breaking off these tubes. Checks of the original suspension were set up at the time of treatment and the total germination in these reached 98 per cent for both fungi. The explanation of the results for the percentage of germination recorded in table 8 is that "Total" gives the figure for the total percentage of germinated spores in the hanging drop, "Growth" refers to the comparative lengths of the germ tubes, and "Killed" is the percentage of *germinated* spores which have been killed by the treatment. Since it has been shown that nongerminated spores are not all killed by treatment, a certain number of nongerminated spores in the suspensions here used must have germinated in the hanging drops after treatment, even though they had been rendered less resistant by their contact with the medium and the cell walls thereby having become softened. This complicated the observation of the number of spores killed, but, by using the mercuric chloride-treated spores as a check, a fair estimate could be obtained, as the germ tubes here had not elongated and collapsed. Where no increase in the length of the germ tubes took place and the amount of germination was the same as in the original suspensions complete killing occurred.

These treatments with germinated spores are duplicates of most of those recorded in tables 6 and 7. Certain similarities are noticed in the results obtained. Naturally mercuric chloride gave complete killing, and also sodium borate gave a greater relative amount of kill of *Penicillium digitatum* than of *P. italicum*. Like in the commercial treatments, the bicarbonates gave a greater relative amount of kill with the former than with the latter fungus. Further, an increase in concentration of treating substances and in time of treatment gave an increase in kill, both of the nongerminated and germinated spores in the mixed suspensions. However, no constant difference in the percentage of kill is noticed between the sodium

and potassium salts, while the same percentage of carbonate is considerably more effective than bicarbonate. This latter effect is undoubtedly due to the high hydroxyl-ion concentration of the carbonate solutions, for it has been shown by many workers that very few, if any, microorganisms can survive in the presence of a pH value above 10. Myers (14), working on the effect of alkaline washing solutions on *Bacterium coli* and *Bacillus cereus,* states that "powders that gave solutions of high pH values were distinctly more effective as germicides than those that gave solutions of low pH values." It is noticed that a 6 per cent solution of sodium bicarbonate, stronger than is used in practice, does not give a satisfactory kill even with the germ tubes exposed to it but that 10 per cent with 10 minutes treatment does.

That the toxicity of the sodium ion plays a very minor, if any, part in the action of sodium bicarbonate is shown by the fact that potassium bicarbonate is just as effective against germinated spores, and the potassium ion has been shown to have no inhibitory power at the highest concentrations used.

DISCUSSION

The whole question of hydrogen- and hydroxyl-ion studies of microorganisms is one which has brought forth many theories and opinions as to the reaction of fungi to these ions. There are, first, the general physiological reactions involved and, second, the specific variations in behavior of different fungi to these reactions. It seems agreed that in the case of acid solutions the inhibitory effect is mainly due to the action of the hydrogen-ion. Boeseken and Waterman (2), assuming that the protoplasm wall in microorganisms consists of an aqueous colloidal solution of lecithins, etc., mixed with albuminoid constituents, refer to toxic action of the hydrogen ion to the precipitation of these colloids. This precipitation may be regarded as a neutralization by the hydrogen ion of the negatively charged plasma colloids, as it were a physical process, and, because all highly dissociating acids act similarly, this explanation has been accepted. Whether the hydroxyl ion acts in the same manner, neutralizing the positively charged plasma colloids, has not yet been suggested, but it is highly probable that in this neutralization phenomenon we have the explanation of the action of the hydrogen and hydroxyl ions, provided they are present in sufficient concentration to overcome the resistance of the living protoplasm. Since the proteins in the protoplasm are amphoteric in nature, it may also be possible that the influence of the hydrogen and hydroxyl ions is such as to reverse the charge on certain proteins, causing a disruption of the protoplasmic system within the cell.

The differences shown by microorganisms in their tolerance to hydrogen and hydroxyl ions are characteristic of the individual species; sometimes

whole genera might react similarly and at other times closely related species might show marked differences. Zeller, Schmitz, and Duggar (29) state that "no general statement, in their opinion, could be made concerning the relation between hydrogen-ion concentration of the culture media and the growth of the wood-destroying fungi as a group." Such variation has been indicated to some extent between *Penicillium italicum* and *P. digitatum* in this work.

Whether germination or growth is used to measure the tolerance of fungi to deleterious substances the general trend of the results is the same, for when the spore wall breaks at the start of germination there is exposed a surface which is just as vulnerable as the tips of the mycelial strands. This would apply only to cases where the deleterious substance is present at the time of actual germination and not to such treatment experiments with nongerminated spores as reported here. The most toxic substances, such as mercuric chloride, kill the spore even before it swells, whereas with hydroxides and carbonates, when present in media of high pH value, a high percentage of the spores swell, even though final germination, as observed by the appearance of germ tubes, is inhibited.

The most striking point in the work with sodium bicarbonate is that its toxic action does not depend on the hydroxyl-ion concentration of its solution, as is the case with strong alkalies. This is shown by the fact that the spores of both fungi germinate in solutions of pH values of 9.0 to 9.6, while the pH of solutions of sodium bicarbonate is from 8.3 to 8.5. That the bicarbonates have an inhibitory power on the growth of fungi has been shown by several workers. It has been shown that enzymes secreted by Penicillium species are instrumental in bringing about the decayed condition of fruits. Nobécourt (15) found, however, that the addition of sodium bicarbonate to the active juice of fruits containing these enzymes makes it lose its property of destroying slices of sound tissue of these fruits. That in the case of Citrus the fruit itself has in no way been conditioned by treatment to resist the growth of the fungi within its tissues is shown by the fact that the blue and green molds do cause a small percentage of characteristic decay even after the fruit has been treated and 100 per cent decay if the rind tissue of the treated fruit is artificially inoculated. The fact remains that the bicarbonate ion in itself has the inhibitory power, and this must be ascribed either to its action on the enzymes or enzyme-secreting power of the protoplasm or to a direct toxic influence on the protoplasm itself. The latter view, in the opinion of the writer, is the more probable.

That the solutions of the hydroxides of sodium and potassium have a more toxic action than the carbonates on nongerminated spores of both Penicillium species was found to be the case upon experimentation. The

former solutions have a much higher pH value than the latter, and if the toxic action is ascribed to the hydroxyl-ion concentration it would be expected that the hydroxides would be more deleterious. The sodium salts, particularly the hydroxide, allowed a lower percentage of germination than the potassium salts, so that it seems that, while the hydroxyl ion is the main factor in inhibiting, the sodium ion assists therein, especially at the higher concentrations of salts.

Coming to the formulation of a theory as to how the actual process of decay prevention takes place the main factor to be borne in mind is that some unkilled spores of the decay-producing fungi are still present on the dry surface of the fruit following treatment. This seems reasonable because a 10 per cent solution of sodium bicarbonate in contact with spores for 10 minutes still allows 85 per cent of *Penicillium italicum* and 35 per cent of *P. digitatum* to germinate. It has been shown, however, that the same solution, almost a saturated one at ordinary temperatures, kills practically 100 per cent of the germinated spores. Further, no germination takes place in solutions of carbonates and bicarbonates plus sucrose, as against 40 per cent in the checks of sucrose in water. Water must be present for the germination of spores on the fruit, and, should water vapor condense thereon and the spores swell and start germinating, an almost saturated solution of bicarbonate will act on them, it being formed from the thin film of the salt left after drying. Death of most of the spores and germ tubes will result, and only those germ tubes can instigate decay which do not come into contact with this solution and which find a break in the rind to enter the albedo. That this percentage is small is shown by the small amount of decay found after mass treatment in packing houses.

It has been suggested that the rind of the fruit absorbs sodium bicarbonate during treatment and its presence therein inactivates the fungus should spores germinate. By making distilled-water extracts of the macerated rinds of well-washed lemons which had been soaked in 2, 3, and 4 per cent solutions of sodium bicarbonate for 24 hours it was found that the pH of the extracts was $\pm$ 5.5, the same as that of the untreated rinds. This pH value would not indicate the presence of free sodium bicarbonate in the rind of treated fruit.

The relation of temperature to the toxic action of the substances worked with here has not been included in these investigations. Weiss (25) showed that an increase in temperature lowered the hydrogen-ion concentration resistance of *Clostridium botulinum*, and the same would hold true for the hydroxyl-ion concentration. A temperature of 100° F. is used for the washing and treating solutions in packing houses, but this temperature *in itself* has no significant killing power on the spores. An increase in temperature increases the speed and probably the penetration power of the

ions, hence a greater toxicity is shown by toxic substances at higher temperatures. Thus it is to be expected that in the treating experiments recorded in this paper a higher percentage of kill would have been obtained by having the treating solutions at a higher temperature, but it is felt that the comparative kills would have remained almost the same. In view of the theory put forward as to the actual process of decay prevention, and, since immersion of fruit in water tends to make the rind slightly more leathery, it is further opined that the real value of warming the water in the treating tanks is that of bringing about a better adhesion of the sodium bicarbonate solution, thereby ensuring a more complete film after drying. This would apply also to treatment with sodium borate.

SUMMARY

In the commercial handling of citrus fruits they are treated before being packed with a 3 per cent solution of sodium bicarbonate for the prevention of the decay caused by *Penicillium italicum* (blue contact mold) and *P. digitatum* (green mold). Investigations as to just how this prevention is brought about, as well as experiments on the hydrogen-ion concentration relationships of the two fungi, are reported in this paper.

By growing the fungi on a Modified Duggar's Solution + orange extract in an apparatus which allowed the daily renewal of the medium under the mats and by weighing the dried mats after 8 days growth, a fairly wide optimum range of hydrogen-ion concentration tolerance for growth was observed, this being pH 2.9 to 6.5 for *Penicillium italicum* and pH 3.0 to 6.0 for *P. digitatum*. No mat at all was formed by the former fungus if the initial pH of the medium was below 2.1 and below 2.4 for the latter. The difficulty in determining the upper limit for mat formation lay in the submerged growth in this range, but the values ± pH 7.9 and ± pH 7.8, respectively, are offered for the two fungi as being near these limits.

Sodium citrate showed a marked inhibitory effect on the growth of *Penicillium italicum* by the sodium ion at a concentration of 20,000 p.p.m. in the medium and on *P. digitatum* at 10,000 p.p.m. The potassium ion has no such effect even at a concentration of 35,000 p.p.m. It is believed that the inhibitory effect on growth exerted by both the sodium and potassium chloride at a concentration of 10,000 p.p.m. of the cations is mainly due to the chlorine ion. The potassium ion does not inhibit germination at the highest concentrations used, but the sodium ion inhibits *P. italicum* markedly at 20,000 p.p.m. and *P. digitatum* at 15,000 p.p.m. Thus the sodium ion is more toxic to the latter fungus.

Certain correlations with the substances used and their toxic action on both germinated and nongerminated spores were observed. An increase in both the concentration of the treating substance and an increase in the

length of time of treatment gave a decrease in the percentage of germination of both fungi. No constant difference was noted in the toxic action of sodium and potassium salts, except perhaps with the treatment of nongerminated spores. The same concentration of carbonate was found to be considerably more toxic than a similar concentration of bicarbonate.

Comparing the effect on the spores of the two fungi sodium tetra-borate was relatively more toxic to *Penicillium digitatum* and the bicarbonates were relatively more toxic to *P. italicum*.

It is believed that the bicarbonate ion as such is toxic to the fungi, for its solution gives a pH value of ± 8.4, and that when the hydroxyl-ion concentration in a solution is large enough to give a pH value of 10+ the toxic property of such a solution lies in the hydroxyl ion, the destruction of the protoplasm being brought about by the neutralization of the positively charged colloids therein or by a reversal of the charge on amphoteric proteins in the protoplasm.

It is further postulated that the manner in which decay prevention is brought about is that when spores of the fungi on the rind of the fruit germinate or start to germinate a saturated solution of sodium bicarbonate formed from the thin film of salt left on the rind after treatment acts on the protoplast of the thin-wall germ tube or on the spore at the spot at which the wall is weakened for the emergence of the tube, death resulting.

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FURTHER STUDIES ON APHID TRANSMISSION OF PLANT VIRUSES[1]

ISMÉ A. HOGGAN[2]

A knowledge of the various modes of dissemination of plant-virus diseases is essential to the development of sound control measures. Insects, particularly aphids and leaf hoppers, are known to play a major rôle in the dissemination of many such diseases, and it appears to be quite generally assumed that tobacco mosaic is spread in this way. As has been pointed out in a recent paper (3), however, the peach aphid, *Myzus persicae* Sulz., one of two aphid species previously regarded as carriers of this disease, does not transmit the true tobacco-mosaic virus[3] between tobacco and other solanaceous plants, although it readily transmits the cucumber-mosaic virus between the same hosts. This, however, does not preclude the possibility of tobacco mosaic being disseminated by aphids of other species. In view of the practical importance of a thorough understanding of the insect relationships of this disease, it was considered desirable to investigate the relation of other species of aphids to its transmission.

It should be stated that tobacco, as grown in the field, does not appear to be a particularly favorable food plant for aphids in the United States. As far as can be ascertained from those familiar with this subject, these insects occur only relatively rarely on the tobacco crop in this country and are in no sense of the word pests on this plant. Nevertheless, it was believed important to determine the behavior of different aphids as potential agents in the dissemination of the tobacco-mosaic disease, and several species, that will feed and multiply more or less readily on tobacco in the greenhouse, have been secured from other hosts. The ability of these to transmit both the cucumber- and tobacco-mosaic viruses forms the subject of the present investigation, the results of which are recorded below.

APHID SPECIES INVESTIGATED

Of the three different strains of the peach aphid which were studied earlier in relation to virus transmission (3), although coming from widely separated localities, none was derived from tobacco while growing in the

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Office of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

[2] This investigation was undertaken on the suggestion of Dr. James Johnson, for whose valuable advice and criticism I wish to make due acknowledgement.

[3] *Tobacco virus 1*, as described by Johnson (4).

field. For purposes of comparison, therefore, further trials have been conducted with two other strains of this aphid derived from such a source, each strain being taken from a single tobacco plant growing in a separate field at Madison, Wisconsin. A third strain, obtained from cabbage in the greenhouse, has also been employed in certain trials.

Other aphid species studied in this investigation and the sources from which they were derived are as follows:

The pink and green potato aphid, *Macrosiphum solanifolii* Ashm.; collected on pigweed, *Amaranthus retroflexus,* on the University grounds, Madison, Wisconsin.

Myzus pseudosolani Theob.; taken from tomato in a greenhouse of the Department of Plant Pathology, University of Wisconsin.

Myzus circumflexus Buckt.; from a potato plant in a greenhouse of the Horticulture Department, University of Wisconsin.

The identifications of the species were kindly made or verified by Dr. A. A. Granovsky of the Department of Economic Entomology, University of Wisconsin.

METHODS

The methods employed in this work were similar to those of the preceding investigation on the transmission of viruses by the peach aphid (3) and need not be repeated in detail.

Stock colonies of each aphid species were maintained in separate insect-proof cages or chambers placed in different greenhouse units. The peach aphid was colonized on cabbage and the remaining three species were maintained on healthy, tuber-indexed potato plants. All transmission experiments were performed in a cool greenhouse in small, insect-proof cages, that were either fumigated or left empty for several days between use for successive trials. Special precautions were taken at all times to avoid any mixing of the aphid species. This greenhouse was maintained at a temperature of 65°–70° F., which proved very favorable for the aphids.

The ability of each species of aphid to transmit the cucumber- and tobacco-mosaic viruses was tested in comparative trials. The aphids were allowed to feed for two to five days on host plants infected with either virus and were then transferred to healthy plants by removing the leaves or stems on which they were feeding and placing them on a piece of paper laid on the new host. Large numbers of aphids in various stages of development were transferred thus to each plant, where they remained two or more days, and were subsequently destroyed by fumigation. In all trials, an equal number of control plants was treated in like manner with mosaic leaves or stems free from aphids. After fumigation, all plants were kept in a warm greenhouse (about 85° F.) in order to favor the rapid development of mosaic symptoms.

The cucumber- and tobacco-mosaic viruses used in the tests came from the same source as those employed in the previous investigation.

EXPERIMENTAL RESULTS

A limited number of trials was conducted with each of the two strains of peach aphid derived from tobacco while growing in the field, in order to determine the capacity of these strains for virus transmission. Several different host plants were included in the tests, the results of which are presented in table 1. These strains proved identical in their behavior with others previously studied. Each was found to transmit the cucumber-mosaic virus very readily between all hosts tested, 100 per cent infection occurring in most trials, while all control plants, with a single exception in the case of strain No. 2, remained healthy. On the other hand, no transmission of the tobacco-mosaic virus was obtained with either strain, whether from tobacco, Physalis, or *Nicotiana rustica*. In short, of the five different strains of the peach aphid which have been studied to date, all have been found to behave alike with respect to the transmission of the two viruses under consideration. The possible dissemination of tobacco mosaic by this species of aphid, at least between the different host plants which have been investigated, therefore appears remote.

Comparative trials have been conducted also of the transmission of the cucumber- and tobacco-mosaic viruses, from both tobacco and tomato, by *Myzus pseudosolani, Macrosiphum solanifolii,* and *Myzus circumflexus.* The results of these trials are summarized in table 2. It will be observed that each of these species resembled the peach aphid in readily transmitting the cucumber-mosaic virus between all hosts tested, *Myzus pseudosolani* and *Macrosiphum solanifolii* causing a particularly high percentage of infection in the majority of cases (Fig. 1). Moreover, there is no significant evidence in these results of any transmission of the tobacco-mosaic virus from tobacco by any species. The 5 tobacco-mosaic infections occurring in the transfers from tobacco, of which 3 were on the test plants and 2 were on the controls, are probably to be accounted for by accidental infection of one kind or another. The highly contagious nature of this mosaic disease should be borne in mind throughout, and, while the number of accidental infections occurring in the greenhouse has been greatly reduced through various precautionary measures in the growing and handling of the plants, it has not been found possible to eliminate such infections entirely in the ordinary routine of greenhouse work.

However, when the tomato plant was used as a source of the tobacco-mosaic virus a singular situation developed. Heavy infection with tobacco-mosaic invariably resulted when *Myzus pseudosolani* was transferred from diseased tomato to tobacco, as high a percentage of infection being obtained

TABLE 1.—*Comparative trials of the transmission of the cucumber- and tobacco-mosaic viruses by two strains of peach aphid (Myzus persicae) derived from tobacco in the field*[a]

Mosaic host (source of virus)	Host	Aphid strain No. 1				Aphid strain No. 2			
		Cucumber-mosaic virus		Tobacco-mosaic virus		Cucumber-mosaic virus		Tobacco-mosaic virus	
		Infested	Control	Infested	Control	Infested	Control	Infested	Control
Nicotiana tabacum	*N. tabacum*	30/29	30/0	30/0	30/0	30/29	30/1	30/0	30/0
Physalis pubescens	*N. tabacum*	5/5	5/0	5/0	5/0				
P. pubescens	*Capsicum annum*					5/5	5/0	5/0	5/0
N. rustica	*P. pubescens*					5/5	5/0	5/0	5/0
Total infections		35/34	35/0	35/0	35/0	40/39	40/1	40/0	40/0

[a] "Infested" denotes those plants to which aphids were transferred from a mosaic host; "control" denotes those plants which were treated with mosaic leaves free from aphids and which served as controls to the infested plants. In all fractions, the numerator represents the total number of plants either infested with aphids or treated as controls, as the case may be; the denominator the number of such plants which developed symptoms of mosaic.

TABLE 2.—*Comparative trials of the transmission of the cucumber- and tobacco-mosaic viruses by different aphid species*[a]

Aphid species	Mosaic host (source of virus)	Mosaic virus	Hosts								Total infections	
			N. tabacum		*L. esculentum*		*P. pubescens*		*C. annuum*			
			Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
Myzus pseudosolani	*Nicotiana tabacum*	Cucumber	50	50	20	20					70	70
			46	1	11	0					57	1
		Tobacco	50	50	20	20					70	70
			2	2	0	0					2	2
	Lycopersicon esculentum	Cucumber	20	20							20	20
			16	1							16	1
		Tobacco	20	20							20	20
			17	0							17	0
Macrosiphum solanifolii	*N. tabacum*	Cucumber	50	50	10	10	10	10	5	5	75	75
			44	0	7	0	7	1	3	0	61	1
		Tobacco	50	50	10	10	10	10	5	5	75	75
			1	0	0	0	0	0	0	0	1	0
	L. esculentum	Cucumber	30	30							30	30
			28	0							28	0
		Tobacco	65	65							65	65
			31	0							31	0
Myzus circumflexus	*N. tabacum*	Cucumber	50	50							50	50
			38	0							38	0
		Tobacco	50	50							50	50
			0	0							0	0
	L. esculentum	Cucumber	30	30							30	30
			22	0							22	0
		Tobacco	30	30							30	30
			13	0							13	0
Myzus persicae	*L. esculentum*	Cucumber	30	30	10	10					40	40
			30	0	8	0					38	0
		Tobacco	65	65	10	10					75	75
			2	0	0	0					2	0

[a] See footnote to table 1.

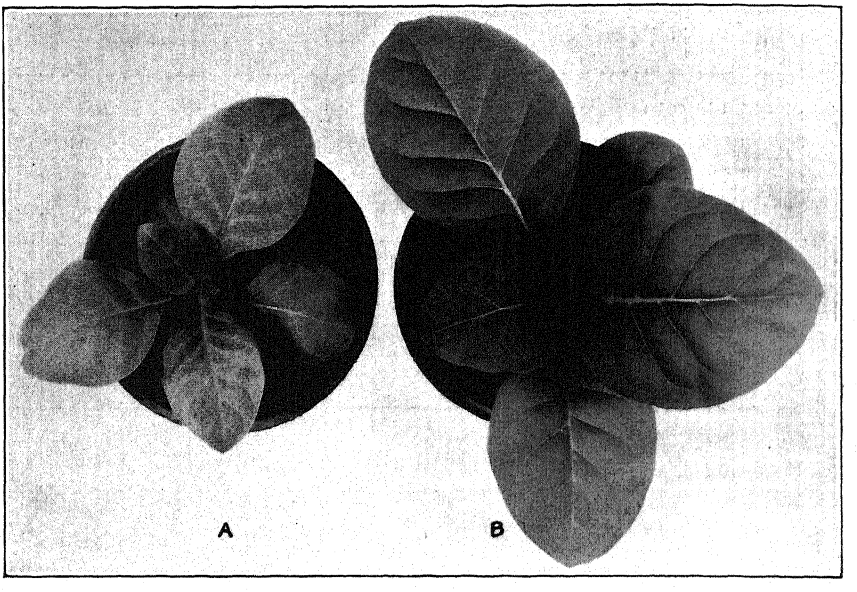


FIG. 1. Tobacco plants showing approximate size and type used in the transmission experiments. A, cucumber mosaic on tobacco, transmitted by *Macrosiphum solanifolii*. B, healthy control plant.

as in the corresponding trials with the cucumber-mosaic virus (Table 2). Since all control plants remained healthy, this infection could not be ascribed in any way to the portions of tomato stems or leaves on which the aphids were transferred, for the control plants were similarly treated. Repeated trials consistently yielded abundant infection in all transfers of this aphid from tomato, leading to the conclusion that in this case the aphid was able to act as a vector of the tobacco-mosaic virus. Subsequent trials with *Macrosiphum solanifolii* and *Myzus circumflexus* showed that these species, too, were able to transmit the tobacco-mosaic virus from tomato, although not from tobacco. With these latter species, however, a distinctly lower percentage of infection was obtained (between 40 and 50 per cent).

In the previous investigation of the peach aphid (3), although a number of different host plants were tested, tomato was not included among these, since it was not considered a satisfactory food plant for the species. The results recorded above, however, rendered necessary a determination of the behavior of this aphid also with regard to the transmission of viruses from tomato, and, by using very young tomato plants and large numbers of aphids, fairly adequate tests could be made (Table 2). The strain of peach aphid employed in these tests was derived from cabbage in the greenhouse. Here, however, repeated trials with the tobacco-mosaic virus gave only two

infections on tobacco in a total of 65 plants, although in corresponding trials with the cucumber-mosaic virus 100 per cent infection was obtained. Whether or not these two tobacco-mosaic infections were due to aphid transmission appears very doubtful, in spite of the fact that all control plants remained healthy. In any case, the peach aphid did not transmit the tobacco-mosaic virus from tomato to any appreciable extent, differing in this respect, at least in degree, from the other species under investigation.

In order to confirm these somewhat peculiar results, more strictly parallel tests were performed with *Myzus pseudosolani,* comparing tobacco and tomato as respective sources of the tobacco-mosaic virus. In these trials, young tobacco and tomato plants were inoculated with virus from a single sample of mosaic-plant extract and, as soon as typical symptoms had developed, were infested simultaneously with large numbers of aphids from the same stock colony. These two host species were isolated in separate insect cages. After several days, the aphids were transferred simultaneously and in great quantities to healthy plants of different species, such as tobacco, *Nicotiana rustica,* etc.; the insects were subsequently destroyed by fumigation and the plants observed for the appearance of disease symptoms. In brief, conditions were exactly duplicated in the two trials, the only difference being in the species of host plant employed as a source of the virus. The results of these trials are presented in table 3. Transfers of aphids from tomato yielded abundant tobacco-mosaic infection on all hosts tested; whereas corresponding transfers from tobacco gave almost no infection. From tomato, of a total of 74 plants tested, 59 showed mosaic infection, with a single infection in the controls; while from tobacco, of a total of 86 plants tested, only 4 became infected, and 1 plant also in the controls. Here, again, it is questionable whether or not the 3 infections occurring on the infested plants only, in the transfers from tobacco, are the result of aphid transmission. The writer is inclined to believe that they are due to accidental infection from other sources, although it is possible that the aphid may have been able, on extremely rare occasions, to transmit the virus from this host. The contrast between the two host species as sources of the tobacco-mosaic virus in relation to the amount of virus transmission is none the less striking. It should be stated that *M. pseudosolani* thrives equally well on both tobacco and tomato; hence these results cannot be accounted for on the basis of suitability of the species as a food plant for the aphid.

The variety of tomato employed in the preceding experiments was Hudson Valley Maid, a potato-leaf variety. Five other varieties have also been tested with respect to tobacco-mosaic transmission by *Myzus pseudosolani,* namely, Marglobe, Bonny Best, Red Pear, Red Cherry, and Vaughan's Model. All these have yielded high percentages of infection, no

TABLE 3.—*Comparative trials of the transmission of the tobacco-mosaic virus by Myzus pseudosolani from tobacco and from tomato*[a]

Mosaic host (source of virus)	Hosts										Total infections	
	N. tabacum		*L. esculentum* (Hudson Valley Maid)		*L. esculentum* (Marglobe)		*N. rustica*		*P. pubescens*			
	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
Nicotiana tabacum	36/2	36/0	20/0	20/0	10/1	10/0	10/1	10/1	10/0	10/0	86/4	86/1
Lycopersicon esculentum	24/22	24/1	20/13	20/0	10/8	10/0	10/10	10/0	10/6	10/0	74/59	74/1

[a] See footnote to table 1.

significant differences in the amount of resultant infection being noted between any of the varieties (Table 4). In all, of a total of 130 transfers

TABLE 4.—*Comparative trials of the transmission of the tobacco-mosaic virus by Myzus pseudosolani from different host species*[a]

Mosaic host (source of virus)	Host	
	N. tabacum	
	Infested	Control
Nicotiana tabacum	36/2	36/0
Lycopersicon esculentum (Hudson Valley Maid)	24/22	24/1
" (Marglobe)	36/33	36/0
" (Bonny Best)	20/17	20/0
" (Red Pear)	10/10	10/0
" (Vaughan's Model)	10/10	10/0
" (Red Cherry)	10/8	10/0
Nicotiana rustica	30/0	30/0
Solanum nigrum	60/7	60/0
Solanum melongena	30/0	30/0
Solanum tuberosum (Green Mountain)	30/0	30/0
Martynia louisiana	30/2	30/0

[a] See footnote to table 1.

of this aphid from tomato to tobacco, 117 plants became infected with the tobacco-mosaic virus, as compared with a single plant in 130 in the control series. Corresponding figures for the transfers from tobacco to tobacco show only 4 plants infected out of 86, with 2 out of 86 in the controls.

As a consequence of these results, it became desirable to investigate the relation of other solanaceous plant species to the transmission of the tobacco-mosaic virus by *Myzus pseudosolani,* in order to determine whether or not the tomato was exceptional in this respect. The following species have been tested: *Nicotiana rustica;* nightshade, *Solanum nigrum;* eggplant, *S. melongena;* potato, Green Mountain variety; also *Martynia louisiana*[4] of the family Martyniaceae. Other hosts, such as Physalis, pepper, etc., were not included since they were found to be unsatisfactory food plants for this aphid. In these trials, little or no evidence was obtained of any tobacco-mosaic transmission by *Myzus pseudosolani* (Table 4). In the transfers from *S. nigrum,* however, 7 plants out of 60 became infected, suggesting an occasional transmission of the virus from this host. Two infections in 30 plants will be noted also in the transfers from *Martynia louisiana,* while from *N. rustica,* potato, and eggplant there was no indication of any virus transmission. Tomato is, therefore, the only host now known from which *Myzus pseudosolani* will transmit the tobacco-mosaic virus with any readiness, and it would appear that this aphid is to be regarded as a carrier of the virus only in so far as certain specific host plants are concerned.

A similar situation has been found with respect to the transmission by *Myzus pseudosolani* of a form of "yellow tobacco mosaic," obtained from a tobacco plant growing in the field at Madison, Wisconsin. This mosaic is apparently identical with that described by Johnson (4) and others, the virus closely resembling in its properties that of ordinary tobacco mosaic. This virus also was found readily transmissible by *M. pseudosolani* from tomato, but not at all from tobacco. In transfers from tomato to tobacco, of 50 plants tested, 35 became infected with yellow tobacco mosaic, while all control plants remained healthy. In corresponding transfers from tobacco, of 50 plants tested, none became infected nor were there any infections on the controls. These studies, therefore, suggest that when determining the ability of certain insects to transmit particular viruses, the species of host plant serving as source of the virus may need to be taken into consideration.

Transmission of the yellow tobacco-mosaic virus by *Myzus pseudosolani* is illustrated in figure 2. This aphid is somewhat peculiar in that it is markedly injurious to the tissues of the host plant, quite apart from any mosaic transmission, presumably due to the presence of some deleterious substance or substances in the saliva. This effect is first seen as a discol-

[4] The writer was informed by Dr. Helen A. Purdy in 1927 that tobacco mosaic was transmissible to this species. This fact has been confirmed and the presence of characteristic cell inclusions (x-bodies and striate material) has been demonstrated in the host tissues.

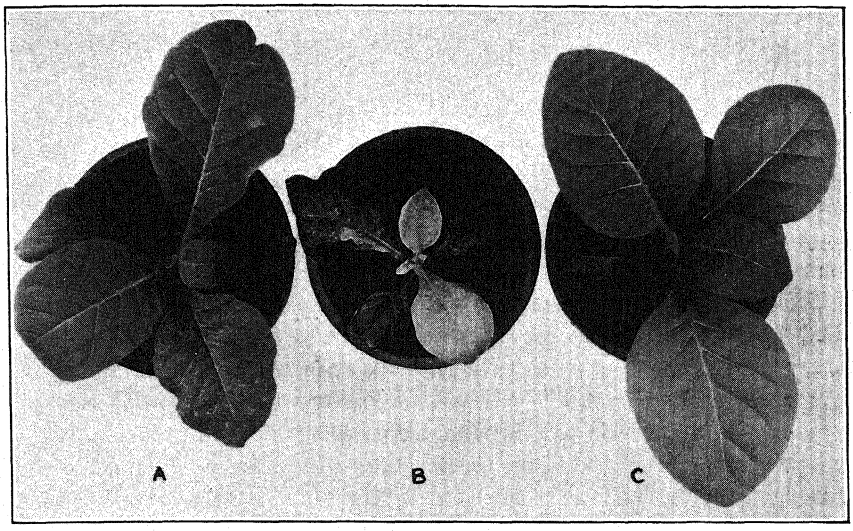


FIG. 2. A, tobacco plant to which *Myzus pseudosolani* was transferred from yellow tobacco-mosaic tobacco, showing aphid injury on lower leaves but no mosaic infection. B, yellow tobacco mosaic on tobacco, transmitted by *M. pseudosolani* from infected tomato. Note aphid injury on lower leaves. C, healthy tobacco plant, control to B.

oration of the tissues in the form of small, pale yellowish spots around the point of entrance of the insect's proboscis; circular on the leaf lamina, and in the form of narrow bands running along the vein, when this is punctured. As the insects continue to feed, the spots enlarge and become golden brown. If the plant is heavily infested, they may coalesce to form large, necrotic areas, frequently resulting in the death of the entire leaf. Eventually the whole plant may be killed. After the aphids have been destroyed by fumigation, the spots may continue to enlarge for a time and the color to intensify, but, if the plant survives, the new leaves develop normally without any spotting, and the plant may eventually completely recover. This injury has been observed on all host plants on which the aphid has fed. It is illustrated on tobacco in figure 2, A. Plant B shows a similar injury, followed by infection with yellow tobacco mosaic, while C is a normal, healthy plant.

DISCUSSION

The development of adequate control measures for the mosaic disease of tobacco is dependent upon a variety of factors. In this connection the particular virus involved naturally requires consideration; however, it appears that, although tobacco is susceptible to a number of different viruses, the ordinary tobacco-mosaic virus (*tobacco virus 1*) (4) is almost universally the cause of the disease so far as field conditions are concerned.

Aphids have been regarded as of major importance in the dissemination of this disease, and *Myzus persicae* and *Macrosiphum tabaci* Pergande have been reported as active carriers of the virus (1, 2). Assuming *Macrosiphum tabaci* to be synonymous with *M. solanifolii,* as is stated by Patch (6, p. 29), it has now been shown that neither of these two species as used in these experiments will transmit the ordinary tobacco-mosaic virus from tobacco, and it seems likely that, where transmission of a mosaic disease of tobacco appeared to be due to these aphids, some other virus affecting this host was actually involved.

Two other aphid species in addition to those named above also have failed to transmit the true tobacco-mosaic virus from tobacco. Furthermore, as has already been pointed out, aphids are not of common occurrence on tobacco in this country. Wilson and Vickery (7) list two species only as reported to occur on this host, namely: *Macrosiphum tabaci* and *Rhopalosiphum* (*Myzus*) *persicae* Sulzer; and, if other species attack the tobacco plant, they do not appear to be abundant. When all these facts are taken into consideration, therefore, the probability of tobacco mosaic being disseminated to any extent by aphids appears remote so far as transmission from tobacco is concerned. Moreover, the occurrence and spread of tobacco mosaic in the field can be adequately accounted for in other ways (5), due in large measure to the highly infectious nature of the virus, together with its remarkable resistance to unfavorable conditions.

On the other hand, since it has been shown that certain aphid species are able to transmit the tobacco-mosaic virus from tomato, it is possible that these insects may be responsible for some dissemination of the disease on tobacco where the two crops are grown in close proximity, and it is not unlikely that they may play an important part in disseminating the disease on the tomato crop, either in the greenhouse or in the field.

Turning to the more fundamental considerations arising from these investigations, the remarkable influence of the species of mosaic host in determining the amount of tobacco-mosaic transmission brought about by certain aphids is deemed worthy of attention. Whether this is an entirely exceptional situation or not is, of course, an open question at the present time. The fact that *Myzus pseudosolani* will so readily transmit the tobacco-mosaic virus from tomato would seem to indicate that there is nothing inherent in the aphid itself to account for its failure to transmit the same virus from tobacco. It would appear, rather, that for some reason the virus is not available to the aphid in the tobacco plant; in other words, that it may be a question of the distribution of the virus within the host. If this is so, then there is no evidence in these studies which would indicate a biological rather than a mechanical relationship between the virus and the aphid vector. Although aphids are believed in general to feed upon

the phloem tissues of the host plant, this may not always be the case, and it is conceivable that these phenomena may be bound up with the nature of the tissues tapped by the aphid in the respective host plants. Further investigations along these lines appear to be necessary before any definite conclusions may be reached.

SUMMARY

1. An investigation has been made of several different species of aphids, in order to determine their ability to transmit the virus of ordinary tobacco mosaic.

2. Two strains of peach aphid, *Myzus persicae,* derived from tobacco, growing in the field, failed to transmit the tobacco-mosaic virus between tobacco and other solanaceous host plants, although they readily transmitted the cucumber-mosaic virus between the same hosts.

3. Three other aphid species, namely, *Myzus pseudosolani, Macrosiphum solanifolii,* and *Myzus circumflexus,* were shown to transmit the cucumber-mosaic virus readily from tobacco and from tomato.

4. The above three species appear unable to transmit the tobacco-mosaic virus from tobacco. They will, however, transmit this virus from tomato, *Myzus pseudosolani* causing very high percentages of infection. Evidence has been obtained to indicate that the peach aphid, on the other hand, does not transmit this virus from tomato, or on only very rare occasions.

5. Transmission of the tobacco-mosaic virus by *Myzus pseudosolani* was demonstrated from six different varieties of tomato, while from four other host species in addition to tobacco no transmission was obtained. From *Solanum nigrum* there was evidence of occasional transmission of the virus.

6. *Myzus pseudosolani* was shown to transmit also a form of "yellow tobacco mosaic" from tomato, but not from tobacco.

7. The evidence thus far obtained indicates that aphids are unlikely to be responsible for any dissemination of ordinary tobacco mosaic, so far as transmission from tobacco is concerned. They may, however, play an important part in the dissemination of this disease on tomatoes, or from tomato to tobacco where these two crops are grown in close proximity.

8. The failure of *Myzus pseudosolani* to transmit the tobacco-mosaic virus from tobacco may be explained on the assumption that the aphid does not extract the virus from those tissues of the tobacco plant on which it feeds.

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LIGHTNING INJURY OF POTATOES

GEORGE F. WEBER

During the past several growing seasons the writer's attention has been called to a specific type of injury of growing potato plants. The injury has appeared in Florida fields where the plants were about two thirds mature, usually during the month of March. After careful consideration of the phenomenon from the viewpoint of a disease caused by a parasitic organism, environmental conditions, physiological disturbances or mechanical injury, together with information supplied by the growers, it was concluded that lightning was the cause of the trouble.

A detailed description of a single occurrence is as follows: During the latter part of March, 1929, an affected area appeared in a 6-acre field of Spaulding Rose potatoes about 70 days old owned by a Mr. Cox near La Crosse, Florida. The field was surrounded on three sides by pine woods and on the fourth side by another field. The affected area was almost circular, measuring 70 by 80 feet in diameter, with the greater diameter parallel with the rows. The soil in the field belonged to the Portsmouth series and was a rather moist, heavy, black sandy loam. The potato plants were in a vigorous condition and were about 16 to 20 inches high with an occasional blossom showing. The injury to the potato plants was not noticed by Mr. Cox until 4 days after the passing of a furious local thunderstorm, which he believes marked the time of the bolt, since showers are rare during the spring and none had preceded this one over a period of 3 weeks. None of the plants were entirely killed and only the plants in a small area, approximately 10 feet in diameter, were prostrate when first observed by the writer 4 days after the thunderstorm (Fig. 1). Outside of this area, none of the plants became prostrate before they were harvested. This condition differs from the case of lightning injury reported by Jones and Gilbert,[1] as they stated that the potato plants were killed in a spot about 12 feet in diameter and that they observed other spots that varied from 8 to 10 feet to 2 to 4 rods in diameter.

Outside of this small area the extent of injury appeared to decrease in direct proportion to the distance from the center, that is, a smaller portion of the stems of the plants had collapsed. For instance, at the periphery of the area, only an occasional growing tip showed any injury, while as one approached the center of the spot more and more of the tops of the plants were injured from the top downward and were shriveled and drooped.

[1] Jones, L. R., and W. W. Gilbert. Lightning injury to potato and cotton plants. Phytopath. 5: 94–102. 1915.



FIG. 1. A. Effect of lightning on potato vines, showing sections of rows of affected potato plants. B. Affected plants in detail, showing collapsed condition with unaffected petioles and leaves.

About half way from center to edge of the spot the plants were affected over half their height from the top down. This is the reverse of the condition reported by Jones and Gilbert,[2] as they stated that "the injured plants still living showed partial collapse and death of the stem from a little below the ground line upward through one half their length, more or less. The tips were living and below ground, the base of the stems, roots, stolons and young tubers seem uninjured." Orton[3] also stated that the stems of the more severely affected plants were collapsed from near the ground upward for several inches and often nearly to the tip.

In the case herein reported no effect of the lightning bolt on the soil in the way of physical disturbances was discovered. A number of hills of potatoes were dug in different parts of the affected area and no marks or signs were discovered on the roots or tubers that could possibly be attributed to lightning.

Furthermore, the stems of the prostrate plants appeared normal below the soil surface; above this point they were shriveled and prominently ribbed and more or less flat (Fig. 2). This collapsed condition included the whole stem from the soil line to the growing tip. The main branches which originated from the main stalk above the soil line were usually not affected but the ones which originated below the soil line were injured. The leaf petioles had also collapsed in some instances, but generally they were apparently unaffected, as they were turgid, brittle, and without unnatural symptoms. Likewise, the leaf blades appeared to be uninjured, even the ones on prostrate stems. The affected plants were green and vigorous, even though they were flattened out and prostrate on the soil surface. In contrast to this, Jones and Gilbert[4] stated that potato plants struck by lightning wilted and died within 24 hours. They also stated that the shriveled stems turned brown progressively above and below the point of injury.

The most peculiar and striking condition in relation to the above situation was that, even though the plants in the center of the spot were so injured as to be prostrate and their stems shriveled and flattened, they still retained their foliage in a healthy, turgid condition indistinguishable from potato leaves outside the injured area.

In order to make a comparison of injured and uninjured stems, specimens were collected within and outside of the affected area. Cross sections were made of the stems of the plants approximately the same distance from the growing tip on injured and uninjured plants that compared favorably

[2] Jones, L. R., and W. W. Gilbert. Lightning injury to herbaceous plants. Phytopath. 8: 270–282. 1918.

[3] Orton, C. R. Lightning injury to potato and cabbage. Phytopath. 11: 96–98. 1921.

[4] *Loc. cit.* 1915.

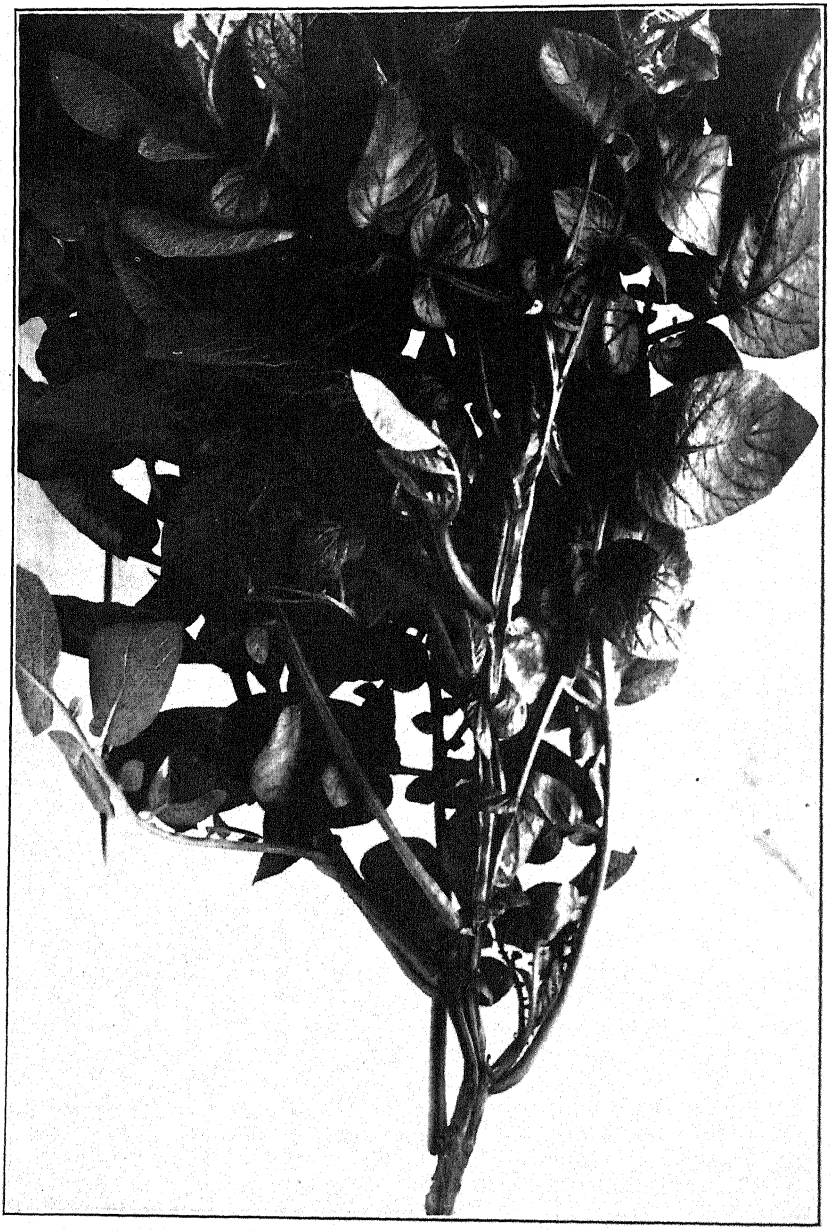


FIG. 2. Single potato stem, showing injury in detail.

with each other in height, diameter of underground stem, and amount of foliage. These cross sections are shown in figure 3. The corresponding parts are designated as (a) epidermis, (b) collenchyma, (c) parenchyma of cortex, (d) pericycle, (e) cambium, (f) vascular bundle, and (g) pith.

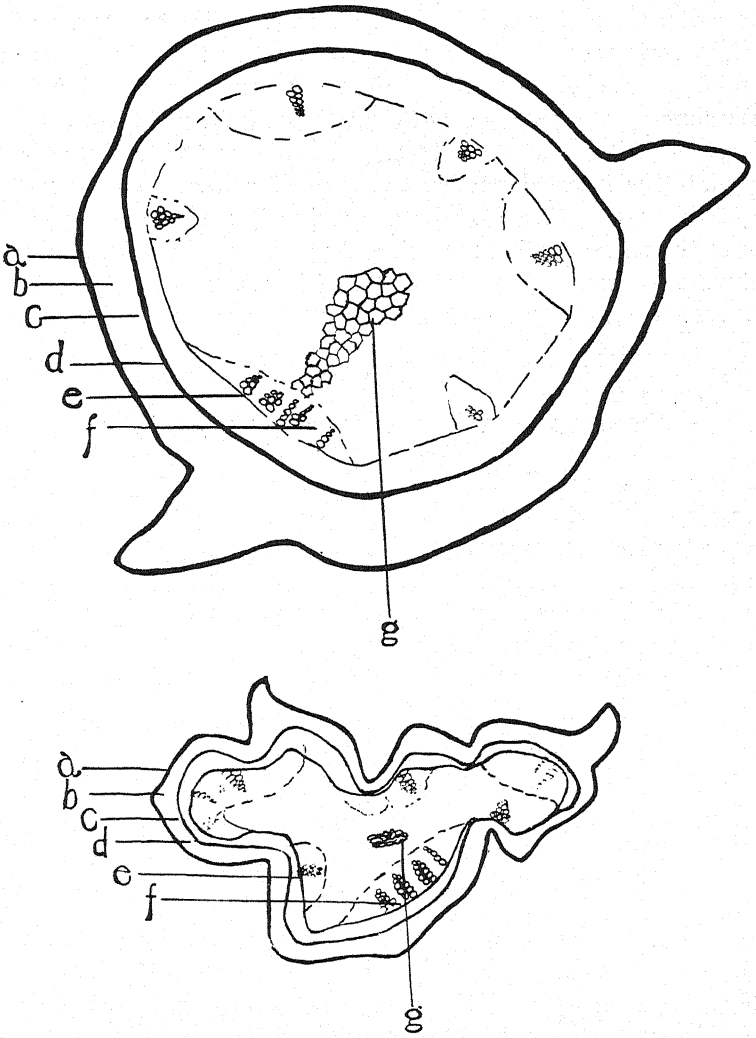


FIG. 3. Cross sections of uninjured (above) and lightning-injured (below) potato stems, showing corresponding morphological characters of each.

a. epidermis
b. collenchyma
c. parenchyma of cortex
d. pericycle
e. cambium
f. vascular bundle
g. pith

The greatest contrast appears in the pith, which has almost totally collapsed in the injured plant. No sign of hollow stem was discovered, as described by Jones and Gilbert[5] in potatoes and by Brown and Gardner[6] in tomatoes. On the other hand, the entire stems of these potato plants collapsed, becoming more or less irregularly flattened. The collenchyma and parenchyma of the cortex also showed a decided collapse, while the vascular bundles occupied very nearly the same amount of area in the injured stems as in the healthy ones, showing that this tissue suffered very little injury. This may account for the turgidness of the foliage of the prostrate plants.

The total collapse of the pith caused the stem to become more or less flattened. The internal portion consisted of a closely compressed tough layer of collapsed pith cells. Upon soaking in water, the cut section, as shown in the figure, tended to swell and partially regained its normal size. It did not fully recover, however, after 24 hours.

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[5] *Loc. cit.* 1915.

[6] Brown, H. D., and Max W. Gardner. Lightning injury to tomatoes. Phytopath. 13: 147. 1923.

PATHOGENICITY OF BACILLUS AMYLOVORUS ON SPECIES OF JUGLANS[1]

CLAYTON O. SMITH

The pear blight organism, *Bacillus amylovorous* (Burrill), a well-known parasite, attacks several species of the Rosaceae. A list of susceptible plants has been prepared by Rosen and Groves (2), but the list includes no hosts outside of the Rosaceae. Burrill (1, 2) and a number of other early investigators reported the occurrence of pear blight on a number of plants, among which were *Juglans cinerea* L., butternut; *Juglans* sp., walnut; and *Hicora* sp., hickory. These early observations were without convincing evidence as to the nature of the maladies. An excellent summary of them has been presented by Snow (4). While no experimental proof has been published of *B. amylovorus* attacking any host except within the family Rosaceae, its successful inoculation[2] on Juglans, as later reported in this paper, would suggest that susceptible plants may be found in other of the botanical families.

The cultures used in this study were isolated from fire-blight lesions of pear trees and a species of Cotoneaster. These cultures, when inoculated

TABLE 1.—*Inoculations on Jugulans with the pear-blight organism, Bacillus amylovorus*

Species	Tissue	Inoculations		Results	
		Manner	Number	Positive	Negative
J. regia	Nuts	Puncture	20	20	0
	Nuts	Contact	10	6	4
	Shoot	Puncture	20	16	4
J. nigra	Nuts	"	20	15	5
	Shoot	"	10	4	6
J. californica	Nuts	"	5	3	2
J. Hindsii	Nuts	"	10	10	0
	Shoot	"	40	34	6
J. Sieboldiana	Nuts	"	20	20	0
	Shoot	"	3	1	2
J. cordiformis	Nuts	"	20	15	5
	Shoot	"	3	0	3

[1] Paper No. 230, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station.

[2] The pathogenicity of *Bacillus amylovorus* on Jugulans was first demonstrated, accompanied by inoculations, by M. C. Goldsworthy (data not published), and was called to the writer's attention about 1926.

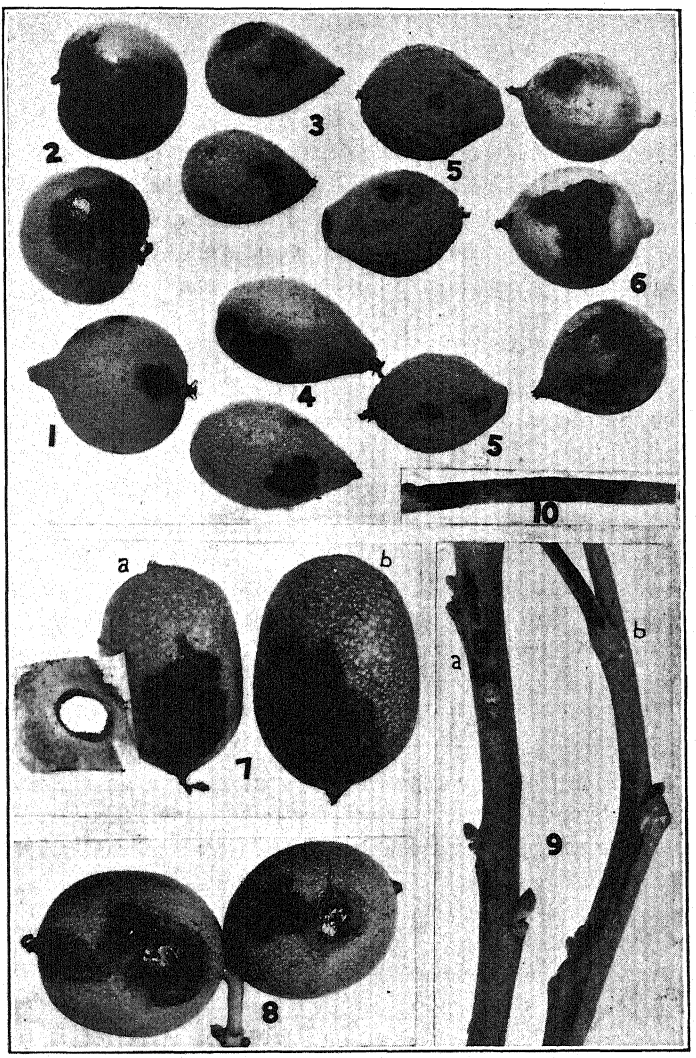
into pear shoots, reproduced the disease in its typical form. The cultures, so far as tested, appeared to be typical of the pear-blight organism.

The inoculation tests were made in 1927 to 1930 on the succulent twigs and, as far as possible, on the nuts of the following species: *Juglans californica* Walt., *J. Hindsii* Sarg., *J. insularis* Grisebach (a Cuban species), *J. major* Heller, *J. nigra* L., *J. regia* L., *J. Sieboldiana* Maxim., *J. Sieboldiana* var. *cordiformis* Makino, *J. mandschurica* Maxim., *J. pyriformis* Liebm. (from Mexico), and some hybrids between some of these species. A partial summary of these inoculations is given in table 1.

INOCULATIONS ON JUGLANS REGIA

The inoculations on the nuts were made by puncture in June and July. Three punctures were made on each of five nearly full-size nuts. In three days from inoculation definite lesions 5 mm. in diameter had formed about the punctures, and in five days the infected areas were 40 to 50 mm. in diameter (Fig. 8). The lesions in most cases had coalesced. The infected necrotic area was darker than normal tissue and eventually had a somewhat water-soaked appearance. The oldest, central part of the lesion was nearly black, while between this and the normal tissue there was a diseased zone of a somewhat deeper green than that of the normal tissue. Some of the infected nuts, when placed in a moist chamber over night, developed sticky drops on the surface, a well-known characteristic of this organism. The tissue of the infected nuts soon showed a shrivelled appearance. With a culture of *Bacillus amylovorus* isolated from *Cotoneaster* sp., the tests were duplicated on Eureka walnuts in 1929, and were repeated in 1930 with the same definite lesions. In these inoculations under field conditions no protection was given the infection courts on the nuts.

The fruits of the English walnut, when inoculated, are apparently as susceptible to *Bacillus amylovorus* as they are to *Bacterium juglandis* (Pierce), a proven pathogene of *J. regia*, and generally ascribed as the sole cause of the destructive disease known as walnut blight. Inoculations by brushing some of the bacterial growth of *B. amylovorus* on the surface of the nuts with a camel's-hair brush, without covering, gave negative results. When the nuts were thus brushed and protected with adhesive tape to which was fastened a circle of filter paper moistened with the bacterial growth, the results in six cases were positive (Fig. 7, also Table 1). In some of the other contact inoculations the results were slight, with some blackening of the surface, or altogether negative. The results are not entirely conclusive that the organism can always enter through the stomata of the nut, but these inoculations would indicate that this sometimes takes place.



FIGS. 1–6, inoculations with *Bacillus amylovorus* on nuts of Juglans. 1, *J. major;* 2, two nuts of paradox hybrid (*J. Hindsii* × *J. regia*); 3, two nuts of *J. Sieboldiana* var. *cordiformis;* 4, two nuts of *J. Seiboldiana;* 5, three nuts of *J. nigra;* 6, three nuts of *J. Hindsii.* 7–8, inoculation on *Juglans regia.* 7a, inoculation by placing bacterial growth on surface and on a circle of filter-paper fastened to adhesive tape and the whole fastened to nut; 7b, nut inoculated like nut at left, only punctured; 8, two nuts inoculated by puncture. 9, shoots of *Juglans regia* inoculated by puncture. Shoot at right shows inoculated petiole and large lesions on succulent growth; shoot at left part of that at right. Note smaller lesions. 10, puncture inoculation on twigs of *Juglans Hindsii.* Photographed after 14 days.

Puncture inoculations on the succulent twigs gave definite lesions (Fig. 9) 15 to 22 mm. long and of a blackish color. On the more woody tissue the lesions were much smaller, as indicated in figure 9, a. These lesions on the English walnut appear to be similar to those reported (5, p. 353) as being produced on this host by *Bacterium juglandis*. While no experimental evidence has been found to show that *Juglans regia* is attacked in nature by *Bacillus amylovorus*, yet such relations should be looked for in future isolations from walnut-blight tissue. Inoculations on succulent pear shoots with *Bact. juglandis* have all been negative.

INOCULATIONS ON OTHER SPECIES OF JUGLANS

The nuts of *Juglans californica, J. Hindsii, J. major, J. nigra, J. Sieboldiana, J. Sieboldiana* var. *cordiformis* and some paradox hybrids (*J. Hindsii* × *J. regia*), also *J. californica* × *J. regia* and Royal hybrid (*J. nigra* × *J. regia*) were inoculated by puncture in the open field with *Bacillus amylovorus*. In 5 to 10 days definite black lesions, 10 to 20 mm. or more in diameter, had developed on all the nuts of the above species, except on *J. nigra*, where the lesions were smaller, about 5 mm. in diameter. (See Figs. 1–6.) This small size is believed to be due to the more mature hard tissue of the larger nuts.

The shoots (Fig. 10) of all these species can be readily infected artificially on the more succulent parts, while the more woody tissue is slightly infected. Probably the slight darkening of some of these latter inoculations extends to little more than the injured tissue. In addition to the above susceptible species, shoots of other species, *Juglans insularis, J. pyriformis*, and *J. mandschurica*, were inoculated with apparently negative results. Inoculations on shoots of *Hicora pecan* Brit. with *Bacillus amylovorus* were negative.

REISOLATIONS AND REINOCULATIONS

Cultures were made from selected artificial inoculations on the nuts and shoots of *Juglans regia* and from them *Bacillus amylovorus* was reisolated. Reinoculations into branches of *Pyrus* sp. gave positive results. The recovery of the organism from the inoculation on other species of Juglans was not attempted.

SUMMARY

Bacillus amylovorus, a well-established pathogene of different species of the Rosaceae, when inoculated into tissues of Juglans, has produced blackish necrotic lesions 5 to 20 mm. in diameter. Under natural conditions the organism has never been demonstrated as attacking any species of Juglans.

The nuts of the seven species, including *Juglans regia* and three types of hybrids between these species, were successfully inoculated, forming lesions not distinguishable from those formed by *Bacterium juglandis*, a pathogene of *J. regia*. The succulent shoots of *J. regia* and most of the other species, when inoculated, proved to be susceptible, forming lesions 10 to 20 mm. in diameter. The epidermis of the shoot was the part attacked and darkened. In the nut the shell seemed to be the part involved.

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TRANSMISSION OF TOBACCO RING SPOT BY SEED OF PETUNIA[1]

R. G. HENDERSON

Reports of seed-borne virus diseases of leguminous plants are not uncommon. In fact, it seems that the virus diseases of legumes are transmitted through the seed in the majority of cases. For instance, McClintock (7), in 1917, reported that lima-bean mosaic was seed-borne. Two years later Reddick and Stewart (10) discovered that bean mosaic was transmitted through the seed, and their results have since been confirmed by the work of Archibald (1), Pierce and Hungerford (9), Fajardo (4), and others. Seed transmission of soy-bean mosaic was reported by Gardner and Kendrick (5) in 1921 and by Kendrick and Gardner (6) in 1924. Dickson (2) increased this list by reporting seed transmission of mosaic for pea, red clover, alsike clover, and sweet pea in 1924.

The brief statement given above will suffice to show that seed transmission seems to be the rule rather than the exception for virus diseases of leguminous plants. This rule, however, will not hold good for the virus diseases of other than leguminous plants. A casual review of the literature reveals the fact that virus diseases of non-leguminous species are very seldom transmitted through the seed, and, in the few cases reported, the amount of infection thus transmitted has been very low in comparison with that reported for the virus diseases of legumes. Doolittle and Gilbert (3), in 1919, for example, reported that out of a total of 130 plants of wild cucumber, *Micrampelis lobata,* grown from seed collected from mosaic-infected plants, only ten developed mosaic. Newhall (8), in 1923, reported seed transmission of lettuce mosaic to the extent of about 3 per cent.

In a recent issue of The Plant Disease Reporter, Valleau (11) reported that evidence had been obtained to show that tobacco ring spot is transmitted through tobacco seed. The results of the writer's studies on tobacco ring spot are not in agreement with Valleau's statement. The writer, however, has found that tobacco ring spot is very definitely transmitted through the seed of garden petunia, *Petunia violacea* Lindl., and the purpose of this paper is to present his data on this subject.

Wingard (12) has already shown that the petunia is susceptible to infection by tobacco ring spot, but natural infection of this host has only recently been observed. The first case of natural infection observed on petunia was found this spring in a flower bed on a Pittsylvania County

[1] Paper No. 76 from the Department of Botany and Plant Pathology, Virginia Agricultural Experiment Station.

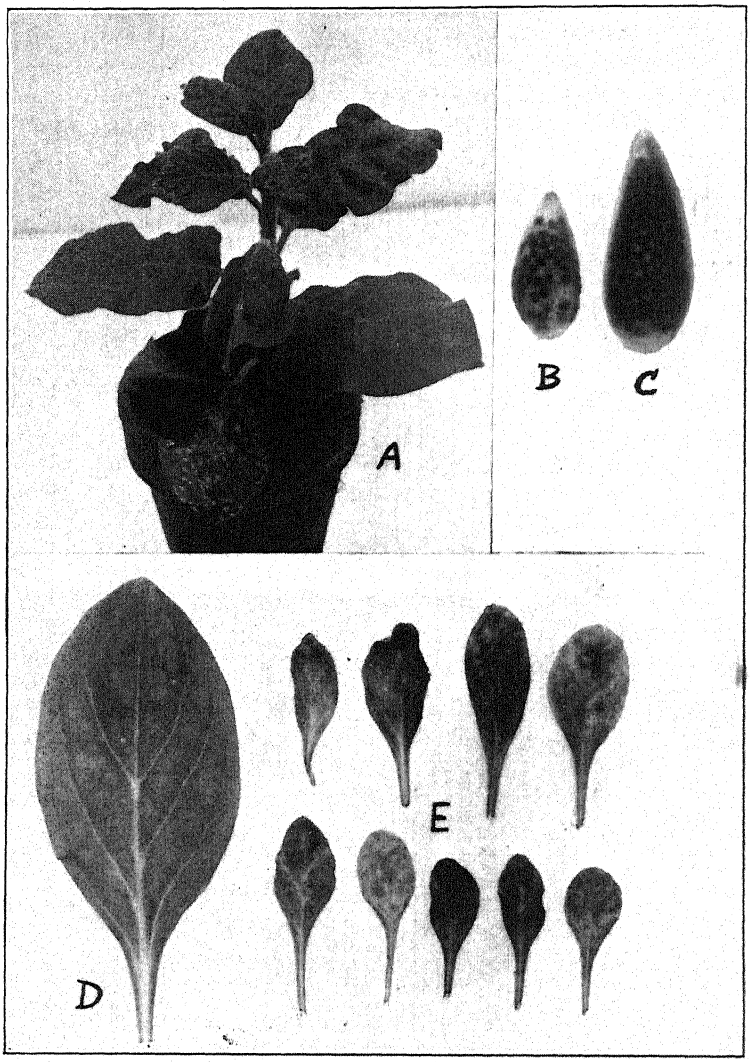


FIG. 1.—A, Turkish tobacco plant 26 days after being inoculated with expressed juice from petunia seedlings infected with ring spot transmitted through the seed. B, half of petunia seed pod from a ring-spot-infected plant. This material was killed in a solution prepared from 5 gm. mercuric chloride, 5 cc. formalin and 5 cc. glacial acetic acid in 100 cc. of 50 per cent alcohol, treated with cellulose acetate solution for about two weeks and finally cleared in oil of Gaultheria. About 2.5×. C, half of a petunia seed pod from a healthy plant, which was treated in the same manner as described above under B. About 2.5×. D, leaf from healthy petunia seedling shown in figure 2, A. About 1.5×. E, leaves from ring-spot-infected petunia seedlings. Some of these leaves were taken from the seedlings shown in figure 2, B. About 1.5×.

(Va.) lawn, and the second instance was found about a month later under similar conditions in Washington County. The symptoms of the disease on the petunia in these two instances corresponded with those described by Wingard for this plant. Of course, the fact that ring-spot infection occurred naturally on petunia in these instances does not necessarily mean that it was transmitted through the seed, but the writer feels that it is a strong bit of circumstantial evidence to that effect.

The writer's experimental results have shown very clearly that the tobacco ring-spot virus is transmitted through the seed of petunia. In August, 1929, a lot of seed was collected from a number of ring-spot-infected petunia plants growing in the Experiment Station greenhouse at Blacksburg, Virginia. These plants had been inoculated with ring-spot virus from tobacco plants. Immediately following the inoculation their growth was greatly retarded, but later the ring-spot symptoms became partially masked and the plants grew and blossomed normally. Many of the flowers, though apparently normal, remained sterile; and those that were fertile set pods that bore only a little seed as compared to a normal pod (Fig. 1, B, C).

About a month after the date of harvest, a portion of this lot of seed was sown in a greenhouse bed. The seed bed had been previously steamed and carefully prepared, and special effort was made throughout the experiment to prevent contamination from outside sources. The greenhouse was kept practically free of insects, and no ring-spot material of any kind was handled near the beds in which the petunia plants were growing. The seed germinated well and the seedlings showed no indication of damping-off or dying from any other cause.

The results of this experiment were quite surprising. Out of the 810 seedlings grown, 160, or 19.8 per cent, developed ring-spot infection. Inoculations with this material gave positive results on tobacco (Fig. 1, A), thereby demonstrating that the petunia seedlings were infected with ring spot. The infected seedlings were severely dwarfed and stunted, as shown in figure 2, B. The first few leaves were mottled in color and streaked with watery green spots and lines that more or less followed the veins (Fig. 1, E). Some of the leaves showed only a few of the watery green areas and many light brown streaks that apparently resulted from a slight necrosis of the leaf tissues. A curling of the leaves along the margin was also noticeable in some cases (Fig. 1, E).

The healthy seedlings were removed from the bed and the infected ones left and allowed to grow to maturity (Fig. 2, D). They remained dwarfed for a short time but later seemed to outgrow the disease and lose nearly all symptoms of infection (Fig. 2, C). Some of the leaves, in spite of the gen-

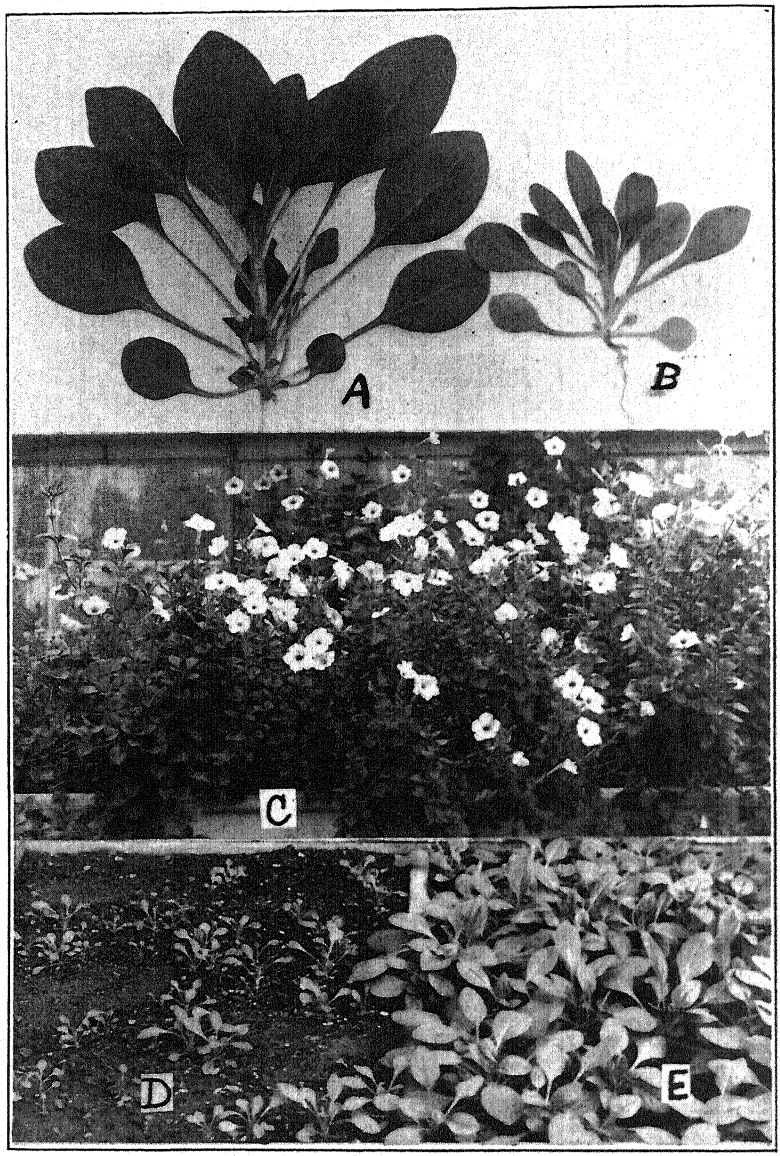


FIG. 2.—A, healthy petunia seedling. About 7/10×. B, ring-spot-infected petunia seedling the same age as the one shown in A. About 7/10×. C, ring-spot-infected petunia plants with the symptoms largely masked. This is the same bed as shown in D and E, but photographed four months later. D, ring-spot-infected petunia seedlings. The healthy plants have been removed. The seed were sown September 24, 1929, and the photographs made November 19, 1929. E, diseased and healthy petunia seedlings. The healthy plants were later removed and the diseased ones permitted to grow to maturity.

eral masking of the symptoms of infection, continued to show streaks, and many developed chlorotic and necrotic rings typical of systemic infection.

On March 24, 1930, the remainder of the lot of seed used in the experiment here reported was sown in the greenhouse. On May 5, an examination was made for ring-spot infection, and 21 out of a total of 104 seedlings exhibited symptoms typical of the disease. This is approximately the same percentage of infection as was transmitted in the previous test, and it shows that the virus did not decrease in virulency during the extended period of time in which the seed remained in storage.

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SOLUBILITY OF BORDEAUX

GEORGE L. HOCKENYOS

Since its first systematic use by Millardet in 1883 Bordeaux mixture has been the subject of much serious study. All phases dependent on the determination of solubility have, of course, been limited to the accuracy of the analytical methods available. Up to the present the most sensitive method of determining copper has been the ferrocyanide colorimetric method, credited with detecting one part of copper in 2,500,000.[1]

A colorimetric method[2] of ten times this sensitivity has recently been published and the writer has applied it to the investigation of some doubtful assumptions now held regarding the solubility of the copper compounds in Bordeaux mixtures.

This method is essentially that originally published and consists of taking 17.5 cc. of the solution to be determined and adding successively 0.5 cc. ammonium hydroxide and 2 cc. sodium diethyldithiocarbonate. The colored solution so produced is compared with a standard similarly prepared in a Duboscq colorimeter. A suitable standard is prepared by diluting 2 cc. of a 1/5000 N solution of copper sulphate to 17.5 cc. and proceeding as above. If the sample is much darker than this it should be diluted and comparisons made between approximately similar solutions.

The writer found that where considerable amounts of calcium salts were in solution there was developed a cloudiness that interfered with the analysis. In such cases the method was modified as follows: To 15 cc. sample add 0.5 cc. concentrated hydrochloric acid to insure a clear solution; then add 1.5 cc. concentrated ammonium hydroxide and 1 cc. of a 1 per cent solution of saponin to peptize the colloids formed and finally add 2 cc. of 1 per cent sodium diethyldithiocarbonate. The standard is made up in the same way and it is essential that approximately similar concentrations of copper be used as the saponin has some color of its own that must be balanced. With the usual precautions attendant with the use of a colorimetric method excellent results can be obtained.

In all of this work the solubility determinations were at room temperature and the Bordeaux mixtures were prepared by dissolving the lime and copper sulphate separately in one half the total amount of water and then pouring the copper sulphate into the lime with vigorous stirring.

[1] Yoe, John H. Photometric Chemical Analysis, Vol. 1, J. Wiley & Sons, Inc., New York, and Chapman and Hall, Paris, 1928.

[2] Callan, Thomas, and J. A. K. Henderson. A new reagent for the colorimetric determination of minute amounts of copper. Analyst 54: 650–653. 1929.

The first point investigated was on the solubility of metallic copper itself. Numerous workers have noted that metallic copper exerts a toxic influence when present in algal, bacterial, or fungous cultures. Nägeli,[3] being unable to demonstrate the presence of copper in solution, formulated the so-called "oligodynamic" theory.

The writer placed some copper wire, which had been cleaned by scraping with a knife blade, in distilled water. Care was used to avoid touching the wire with the hands. One sample was placed in water recently boiled to free it of gases and another in water saturated with atmospheric gases by bubbling washed air through it. The containers were then tightly corked. The percentage of copper in solution is shown in the following table:

	Boiled Water	Aerated Water
24 hours	0.00013 per cent	0.00006 per cent
3 days	0.00039 " "	0.00032 " "
9 days	0.00104 " "	0.00039 " "

The fact that more copper, ultimately dissolved in the boiled water, may be associated with the formation of an insoluble film in the presence of dissolved atmospheric gases.

Bordeaux is commonly made on a formula approximating the so-called 4–4–50 or 4–6–50 formula. These latter two formulae give 1 per cent copper sulphate to 1 per cent lime or 1 per cent copper sulphate to 1.5 per cent lime, respectively. Pickering,[4] in 1917, published a list of the compounds formed when copper sulphate and lime are mixed in various ratios varying from 1 part copper sulphate to 0.169 parts lime up to 1 part copper sulphate to 0.67 parts lime.

A series of seven mixtures were made up, five of them in the ratios indicated by Pickering and the other two in the ratios commonly used in orchard practice. In all cases 1 per cent copper sulphate was used. Thus in the case of sample F the solution was equivalent to a 4–4–50 Bordeaux. The percentage of copper in the supernatant fluid was determined after three days' settling.

In their book, "Science and Fruit Growing," Spencer and Pickering discuss these formulae as follows: A is just enough lime to precipitate all the copper present, C is a neutral Bordeaux, and, if excess lime is used, an

[3] Nägeli, Carl K. von. Ueber oligodynamische Erscheinungen in lebenden Zelle. 51 pp. 1893. Zürich.

[4] Pickering, S. V. The Chemistry of Bordeaux mixture. Jour. Chem. Soc. 91: 1988–2001. 1907.

	Ratio copper to hydrated lime	Orchard formula	Pickering's formula	Per cent copper in solution	Color of precipitate
A	1–0.169	4–0.67–50	10 CuO 2.5 SO_3	0.04	Greenish
B	1–0.18	4–0.72–50	10 CuO 2 SO_3	0.03	"
C	1–0.203	4–0.81–50	10 CuO SO_3	0.016	Greenish-blue
D	1–0.27	4–1.08–50	10 CuO SO_3 3 CaO	0.00009	Pale blue
E	1–0.67	4–2.68–50	10 CuO 30 CaO	0.00013	" "
F	1–1.0	4–4–50		0.00014	Medium blue
G	1–2.0	4–8–50		0.00014	Dark blue

alkaline Bordeaux results with compounds C and D being formed and it is only after several days standing that compound E is formed. They further add that in the case of formula D no copper can be detected at all in solution.

In making the above analyses the writer pipetted a portion of the supernatant liquid, rather than filter it off, because it was found that filtering altered the copper content of not only the Bordeaux solution but of the standard as well. This may have been due to the absorption of the copper ions by the filter paper or to the interference of filter-paper fibrils in the formation of the colored compound. That the copper obtained in these analyses was not particles in suspension is shown by the fact that a precipitate made by formula F was repeatedly decanted with distilled water and finally washed on a filter. Upon being shaken with distilled water and allowed to stand two days, it gave 0.00013 per cent copper in solution. A further check was made by drying such a precipitate in the oven over night at 180° F. and then shaking in distilled water. After two days an analysis showed 0.00010 per cent copper in solution.

In commercial practice, stone lime or calcium oxide is sometimes used in place of the hydrated lime or calcium hydroxide. Several mixtures were made up using the chemical equivalent of stone lime in place of the hydrated form. The lime was boiled in a small amount of water and diluted before mixing with the copper sulphate. This gave a very milky lime suspension and when precipitated it gave a dense dark blue precipitate. The liquid, however, tested 0.00010 per cent copper and did not differ greatly from the hydrated lime precipitates. It was also found that if hydrated lime be boiled vigorously for a few minutes with a small amount of water before diluting and mixing with the copper sulphate solution, it tends to make a darker colored and denser precipitate than where simply shaken up in the cold.

Sugar has frequently been used as a preservative and it has been suggested that its action is due to its chemical combination with the calcium

hydroxide. Certain it is that both the precipitate and the supernatant liquid take on a deep blue color when sugar is added. A solution containing 0.5 per cent sugar was analyzed and found to contain 0.078 per cent copper actually in solution.

Numerous other applications of this method suggest themselves. Among these are the effect of atmospheric agents on Bordeaux spray on fruit-tree foliage and the effect of the excretions of the leaves themselves. Some studies of these factors were recently made by DeLong[5] but this method was not used.

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[5] DeLong, D. M., W. J. Reid, Jr., and M. M. Darley. The plant as a factor in the action of Bordeaux mixture as an insecticide. Journal Economic Entomology 23: 383–390. 1930.

THE AVOCADO DISEASE CALLED SUN BLOTCH[1]

WM. T. HORNE AND E. R. PARKER

The writers are not aware that the avocado disease called sun blotch occurs in any country except California.[2] We do not have accurate information as to when it was first observed in this State, though it is now frequently met with and may be considered of general distribution here.

The first published mention of the disease which has come to our attention is the article by Dr. Coit[3] where a brief, but effective, description of the disease is given with five illustrations. The nature of the disease is indicated and an interesting hypothesis set forth as to its cause, and, finally, the most important procedures as to control are pointed out.

As indicated by Dr. Coit, symptoms of sun blotch involve the fruit, young stems, branches in general, and trunk. On the fruit, long and narrow, shallow, longitudinal grooves or depressed streaks appear near the stem end (Fig. 1). The surface of the streak tends to be smooth. If the fruit is short and broad the streak tends to be a smooth, flattened or somewhat depressed area radiating from the vicinity of the stem end. The color of the fruit streak in green fruits is whitish or yellowish, whereas with purple varieties affected areas develop striking red or purple red colors.

On the young stems shallow, light-color or, in some varieties, buff-color longitudinal grooves or streaks may appear (Fig. 2). Some bright red streaking may show also in the stem lesions. Where symptoms are well developed they are striking, but frequently the streaks fade out to merely obscure mottling; also excessively vigorous normal shoots have certain longitudinal grooves and ridges. As heavily affected stems become somewhat older they become very uneven and rough with prominent lenticels. The shoots become decumbent, somewhat twisted and abnormal in appearance, having a dull color and unthrifty aspect. In addition to the symptoms described by Dr. Coit we have observed on the variety Caliente a white variegation of unequal distribution on the leaves. This appears to be a symptom of sun blotch. Bark of the trunk of sun-blotch trees has been observed to be unusually rough, but we are not sure that this is an invariable indication of the disease.

[1] Paper No. 228, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Dr. H. S. Fawcett, in a personal letter written from Rome on June 11, 1930, states that he saw sun blotch in a tree or two in Palestine, trees which he understood came from California.

[3] Coit, J. E., Sun blotch of the avocado, a serious physiological disease. Yearb. Calif. Avocado Assoc. 1928: 27–32. 1928.

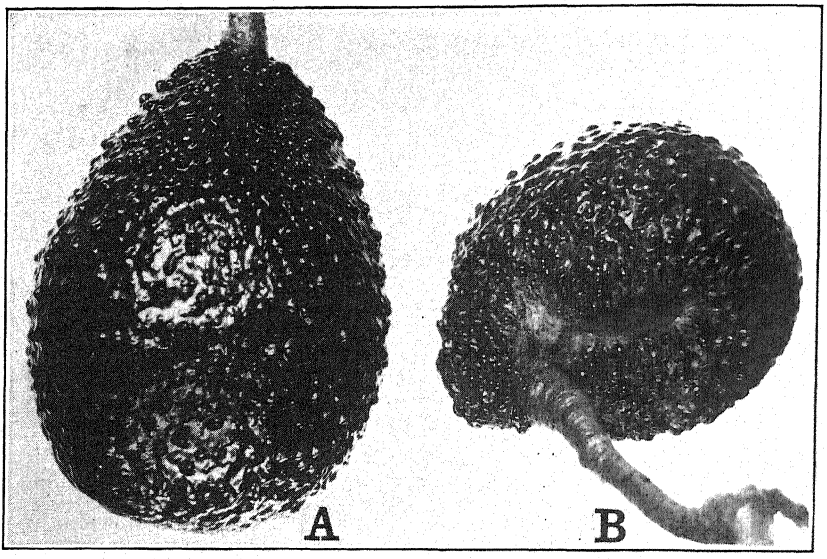


FIG. 1. Sun blotch in avocado fruits of the Lyon variety. A, a pronounced longitudinal crease near the stem (see arrow) which is sufficient to disqualify the fruit for first grade. B, broad, deep depressions near the stem, causing serious distortion. In one of the sun-blotch depressions is an area of secondary necrosis.

A striking feature appears to be the variation in the degree to which symptoms are shown. Some trees have undoubted symptoms of the disease yet appear normal, except on close examination, and it is probable in some cases that affected trees cannot be recognized even by experienced persons. In other cases affected trees become very abnormal and worthless. Severe pruning appears to accentuate symptoms on the robust shoots. It seems probable that the appearance of symptoms of the disease and their severity are related to the vigor of growth of the affected part.

The disease has been observed on numerous varieties and none are known to be immune. Importance of the disease is as yet uncertain. If it should develop extensively in its more severe form, as it appears on unthrifty nursery trees, it would certainly be disastrous. On some large trees the only injury discoverable is an occasional slightly marked fruit which becomes a cull, general vigor and production being fairly normal.

In the affected parts of the plant there appears to be a deficiency in the development of certain tissues but the pathological histology has not yet been worked out.

The suggestion made by Dr. Coit as to the cause of sun blotch, *i.e.*, that it originates where tissue is injured by sunburn, is interesting. Whatever the origin, it seems clear that sun blotch, once originated, may be continued

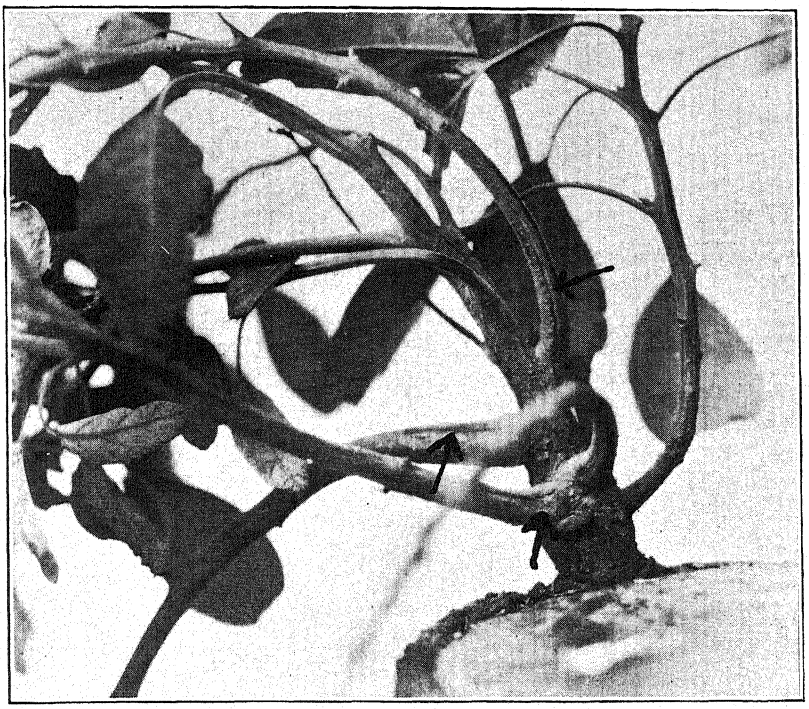


FIG. 2. Sun-blotch scion on healthy stock showing beginning of the decumbent habit, grooves on the twigs (see arrows), and rough abnormal bark.

by budding or grafting and is then not dependent on sunburn for its development.

It is well recognized by practical men that a sun-blotch scion set in a normal tree grows with the sun-blotch character. Scions set by the second writer at the University of California Citrus Experiment Station, Riverside, Calif., have shown this behavior. In the case of a tree with only slight symptoms of sun blotch, we are not sure that all twigs would, if used as scions, transmit the symptoms to shoots originating from them.

Dr. Coit gave the writers certain evidence indicating that where a sun-blotch-diseased scion grew on a healthy stock shoots subsequently arising from the stock (Fig. 2) also might show the disease. In at least three cases this has clearly occurred at the Citrus Experiment Station. The disturbed condition, therefore, progresses from diseased scion to healthy stock. Growers state that healthy scions set in sun-blotched trees grow out with the symptoms of sun blotch. Owing to the difficulty involved in making sure that a given scion is free from sun blotch, this point is receiving further study.

Inoculations on seedlings have been made in several ways: by needle pricks, first moistening the needle with juice of affected tissue; by hypodermic needle, introducing juice from diseased tissue; by hypodermic needle, using juice from diseased tissue which had been passed through a Berkefeld filter; and by introducing pieces of living diseased tissue. Results from these inoculations are negative to date. Through the kindness of Professor E. B. Babcock a set of plants was treated with X-rays, but it is not yet apparent that any permanent change in the tissues has been induced.

From the above evidence it would appear that the disease of avocados called sun blotch belongs to the group of plant diseases called infectious chloroses.

DOWNY MILDEW OF SORGHUM AND MAIZE IN EGYPT[1]

L. E. MELCHERS[2]

The downy mildew of sorghum and maize was found by the writer in June, 1928, on the agricultural experimental farm belonging to the Ministry of Agriculture, Giza, Egypt. It had not previously been reported in Northern Africa. The disease was first noticed on plants of sorghum (durra) as they approached the heading stage in a "date-of-planting" experiment which had been made for the purpose of studying the effect of soil temperature on kernel-smut (*Sphacelotheca sorghi* (Lk.) Cl.) infection. All plantings became attacked by the downy mildew. Later, the disease appeared on some maize which grew adjoining the durra plots.

As this was the first time downy mildew had been observed in Egypt, the matter was of considerable importance because of the place which durra, and especially maize, hold in Egyptian agriculture. The various infected plantings were immediately ordered removed and burned, and close watch was kept on all other plantings in the vicinity. Downy mildew appeared later in another section of the farm on some sorghum varieties, the seed of which the writer had brought from Kansas. All plants were removed as soon as they were found to be infected. Several scouting parties were sent to other Provinces where sorghum (called millet in Egypt) is grown to search for the presence of downy mildew. Fortunately, it was not found at that time on sorghum or maize in any other Province except at Giza.

The source of the infection by this fungus is not definitely known. Circumstantial evidence indicates that it may have entered Egypt on packing materials from India. The Section of Horticulture at Giza imports plants from India and these may have been the source of the inoculum. The writer examined the grasses in the vicinity of the Giza farm, believing that infection very probably originated from such a source, but no evidence of its presence on grasses was discovered. It is possible that if additional time had been given to a more comprehensive search of the wild grasses of the tribe Andropogoneae of the Gramineae, its presence would have been found; at least, this is the logical place to have expected its occurrence. The entire matter of the origin of the fungus in Egypt remains problematical.

During the latter part of June, and July, August, and September, when maize and sorghum are growing, the climate of the delta is generally warm

[1] Contribution No. 309 from the Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station.

[2] Chief Mycologist, Egyptian Ministry of Agriculture, Cairo, Egypt, 1927 to 1929.

and humid; therefore, conditions are favorable for the development and spread of downy mildew.

Material was examined microscopically by Dr. R. M. Nattrass, one of the assistants of the Mycology Division, who identified the organism as *Sclerospora graminicola* var. *andropogonis sorghi*. Examinations by Dr. Nattrass and myself of the downy mildew on maize led us to believe that it was the same species. No striking differences in appearance or measurements could be found between the conidial stages on durra and on maize. Apparently it is the same species as that present on sorghum in India. If it is the same species on sorghum and maize in Egypt, the matter is of considerable scientific interest, as all literature describes the species attacking maize as an entirely different species and there seems to be no record of the same species attacking both maize and sorghum.

Upon returning to the United States, the writer sent specimens on sorghum to W. H. Weston, Jr., who kindly examined the material and compared it with specimens from India, Java, and South Africa. As a result of his examinations he has informed the writer that he would identify the Egyptian form on sorghum as *Sclerospora graminicola* var. *andropogonis sorghi* of Kulkarni. According to Dr. Weston the conidial stage present in this Egyptian material is not sufficiently mature to show certain features important in diagnosis, but the resting spores (oogonia with oospores) are fairly abundant and well developed. These bodies, in diverse collections of this Sclerospora from sorghum, show some variation. In essential characteristics of size, structure, color, etc., this Egyptian material most closely resembles the specimens from Kirkee, India, rather than those from Poona, India, where Kulkarni originally established the variety.

A more complete ecological study of this problem in Egypt would have been extremely interesting, together with cross-inoculation studies on durra and maize. This was not attempted because of attending dangers. Every effort was made to eradicate this disease as soon as it was discovered, as there was no way of knowing how rapidly it might spread. In the writer's opinion, there is circumstantial evidence to indicate that downy mildew may have been present on sorghum plantings at Giza in 1926 but was not recognized as such by the mycologist in charge. Although every precaution was taken to prevent this disease from becoming established in Egypt, there is some doubt whether downy mildew of sorghum or maize would find the proper environmental conditions to produce epiphytotics in that country.

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PHYTOPATHOLOGICAL NOTES

Another host for Ustilago striaeformis (Westd.) Niessl.—In May, 1929, the writer found a plant of bottle-brush grass, *Hystrix hystrix* Millsp., heavily infected with a leaf smut. The plant was growing in a yard at Manhattan, Kansas, and undoubtedly was from seed scattered by a plant that had been grown, the preceding season, as an ornamental in the garden. Many other seedlings of the same species were growing within a few feet of the smutted plant, but none of them showed any infection.

Leaf smuts of grasses are seldom seen in the vicinity of Manhattan, so the infected plant was carefully transplanted to a pot in the greenhouse where it could be watched. Here it grew to normal maturity and produced a spike typical of *Hystrix hystrix*. When found the plant had only four or five leaves, all of which, except the first two, showed the long grayish black lesions characteristic of this leaf smut on other grasses. All subsequent leaves showed many such lesions. As is typical of most leaf smuts on grasses, the sori at first were covered by the epidermis, but as the leaves matured, the epidermis ruptured and exposed the black spore masses.

At maturity the smutted leaves were collected and a specimen sent to the Office of Mycology and Disease Survey, United States Department of Agriculture, where the fungus was identified by R. W. Davidson as *Ustilago striaeformis* (Westd.) Niessl. Another specimen was sent to Dr. A. G. Johnson of the Office of Cereal Crops and Diseases who, together with Mr. W. W. Diehl, also identified the fungus as *U. striaeformis*. Reports from both sources stated that no previous record of the occurrence of *U. striaeformis* on *Hystrix hystrix* could be found. Therefore, it seemed desirable to record *H. hystrix* as another host for this smut. The other plants of this grass, growing within a short distance of the smutted plant, were observed at frequent intervals throughout the summer of 1929, but none of them developed the smut. Seedlings from those plants emerged in the spring of 1930, but, out of more than 100, none have shown signs of leaf smut.—C. O. Johnston, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the Kansas Agricultural Experiment Station, Manhattan, Kansas.



William Harmon Wright

PHYTOPATHOLOGY

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WILLIAM HARMON WRIGHT
1885–1929

E. G. HASTINGS

At the beginning of the present century the passing of a young man from life on a farm to the university of his State was something accomplished only after serious thought and under some special stimulus. A study of the various stimuli that have been active in attracting young men out of the road that stretched away so clearly before them, the road of their normal environment, to a road up which they could see only the shortest distance, which ended in conditions no one could predict, would prove interesting. For a prominent geologist of the writer's acquaintance, the stimulus was a tray of minerals that in some way or another reached his boyhood home; for a chemist, the formulae on the sacks of fertilizer used on the farm. Probably the lure of amateur snapshots drew W. H. Wright from a farm in southern Indiana to study at Purdue University in 1904. But the stimulus that attracted him to a life of teaching and research in biology is most evident. It was Dr. Stanley Coulter, for a long period in charge of biology at Purdue University. Dr. Wright transmitted this stimulus to many young people. Thus the influence of the teacher is an enduring and ever-widening one.

Dr. Wright graduated at Purdue in 1908, and in the fall of that year came to the University of Wisconsin as a graduate student in bacteriology and chemistry. There he received the degree of Master of Science in 1909. In the fall of that year Dr. Wright joined the staff of the Department of Agricultural Bacteriology as an assistant. He was promoted through the various ranks and at the time of his death, May 3, 1929, was associate professor of Agricultural Bacteriology. He had thus almost completed two decades of service in the University.

His residence at Madison was broken by one year of graduate work at Cornell University, where he studied under the late Prof. W. A. Stocking. In 1925 he was granted the degree of Doctor of Philosophy by the University of Wisconsin.

The period of Dr. Wright's service included the years when agricultural colleges reached the peak of their enrollment. There were many students to teach and a large part of this load fell to Dr. Wright. At first the teach-

ing was chiefly in connection with the laboratory work of the elementary courses; later, lecture work with short-course students in agriculture and classroom instruction in the college courses in beginning bacteriology, and, finally, with the development of a course in the special technique of the bacteriological laboratory. All these various types of work were carried with a superlative degree of success.

To some, the acquaintance of the teacher with his specific subject and with its relation to other subjects and his ability to present the facts and relationships in a vivid manner, to have enthusiasm for his work and to impart it to some of his students, to be willing to enter into the school life and the extra-school life of his young people, and to share his time and energy in and out of class-room and laboratory seem more important than fine-drawn definitions and excessive theory in teaching. I am not aware that Dr. Wright ever took a course in education, but I am certain that he had and used the qualities of a great teacher.

His ingenuity and his bent toward things mechanical helped him to develop a course in bacteriological laboratory technique, which many graduate students have asserted to have proven their most valuable course. It was this characteristic which attracted him to a study of the mechanical isolation of single bacterial cells, suggested and used by Barber two decades ago, and to suggest certain changes in the technique which made the method more certain. Before one can assert that a particular organism possesses a specified characteristic or that a certain culture represents an intermediate strain between two types of pathogenic organisms the purity of the culture must be certain. The single-cell technique enabled Dr. Wright to answer some of the questions which bothered the student of crown gall and hairy root. Known pure cultures fell into one or the other group. The intermediate strains that had perplexed the plant pathologist disappeared, and the confusion was shown to be due to mixed cultures obtained by the usual plate method. Still a third organism, *Bacillus radiobacter*, entered still farther to confuse the student of this problem in phytopathology. The single-cell technique supplied once more clarity in place of turbidity. The researcher in this field now has available a method of determining whether the niche that will permit him to climb a bit higher is a safe one or not. Safe technique faultlessly used is a necessity to all progress. In perfecting and using such technique, Dr. Wright made important contributions to progress in plant pathology as well as to progress in other lines of bacteriology.

The field of variation among the legume bacteria and of their varying effect on the host plant, noted by Hiltner and Störmer, was reexamined by Dr. Wright, especially as regards the soy-bean nodule bacteria.

At the time of his death he was actively engaged in a study of the single-cell technique as applied to certain human pathogenes and in a study for the Bureau of Fisheries of the causes of the deterioration of fish nets and of means of delaying their disintegration, a phase of the great field, still an almost unknown one, of the decomposition of cellulose.

Doctor Wright's interest in everything that life presents made him a valuable member of his home and college community; made him an enthusiastic cooperator in all efforts to make, in every way, life more beautiful and more enduringly satisfying.

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SPUR BLIGHT OF RASPBERRIES IN ONTARIO CAUSED BY DIDYMELLA APPLANATA[1]

L. W. Koch

INTRODUCTION

Spur blight is a fungous disease of raspberries, so called because of its habit of attacking the spurs or laterals of the canes and partially or completely destroying them. It has often been referred to as "cane blight" in America and quite consistently as such in Europe. In Ontario "spur blight" is the preferable name because here a quite different fungus is responsible for symptoms of a disease commonly referred to as cane blight.

Spur blight is prevalent in the Niagara Peninsula and is gradually becoming a problem of importance to the growers of that district. Its increasing prevalence, coupled with the meager amount of literature dealing specifically with the disease, makes spur blight a problem of scientific interest and economic importance.

HISTORY AND GEOGRAPHIC DISTRIBUTION

In America spur blight was evidently first reported by Miss Detmers (8) in 1891. She attributed the brown discolored areas on the canes to bacteria, as did also Card (5). Peck (34, p. 114) found a fungus associated with spur blight and, in 1894, first described the perithecial stage of the causal organism in America. He attributed it to a species not previously described, namely, *Sphaerella rubina* Pk. Stewart and Eustace (46), in New York, attempted to obtain pure cultures of *S. rubina* by the dilution method in 1902. They state that they were unsuccessful owing to the frequent occurrence of a fungus developing Phoma pycnidia in the cultures. It seems probable that these workers were obtaining the imperfect stage in their cultures but did not interpret it as such. They also found a *Coniothyrium* sp. commonly associated with the spur-blight perithecia.

Since that time spur blight has been reported by various writers from widely separated regions in the United States and Canada. Sackett (41), in 1915, published an article in Colorado dealing with spur blight in which he described the symptoms, the perithecial stage as described by Peck, a

[1] These investigations were begun in the spring of 1927, at the Dominion Laboratory of Plant Pathology, St. Catharines, Ontario, where the work was continued during the summers of 1927, 1928, and 1929. During the fall and winter of each of the intervening years, the investigations were continued in the Department of Botany, University of Toronto. My thanks are due to Dr. G. H. Berkeley, of the St. Catharines laboratory, to Professors D. L. Bailey and H. S. Jackson, of Toronto, for their advice and criticism, and to the National Research Council for the bursary granted by the Council in 1928–29.

few pathological changes, and several control experiments carried on in Colorado. Newhall (27), in 1923, inoculated young raspberry canes with ascospore suspensions of *Sphaerella rubina* and observed that a *Phoma* sp. developed in the consequent lesions. He also established in pure culture the connection of this species with the spur-blight perithecia. Zeller and Norris (53), in 1925, and Zeller (54), report it to be very common in Oregon and throughout the Pacific Northwest. According to Colby and Anderson (6) spur blight is quite generally distributed in the United States and has been reported from widely separated regions. Dodge and Wilcox (9), in 1926, say that spur blight has recently been reported to be serious in certain localities in the Eastern and Midwestern States. Anderson *et al.* (1, pp. 92–93), in 1926, state that spur blight is widespread on raspberries in the Northern States and Zeller (52) reported the disease in Washington on the loganberry. Other reports of spur blight in the States come from Robbins and Reinking (38), Bennett (3), Daniels (7), and Hall (16). In Canada, Drayton (10), in 1926, reported spur blight common in Manitoba, Ontario, Quebec, Nova Scotia, and Prince Edward Island.

IN CONTINENTAL EUROPE AND ELSEWHERE

The earliest reference to the spur-blight fungus in Europe was that of Niessl (29) in 1875. He assigned the perfect stage of the fungus to *Didymosphaeria applanata* (Niessl) Sacc. It was later transferred by Saccardo (40) to Didymella and this name is the one usually used in European literature. Since 1875 numerous writers in different countries have referred to the disease, though few workers have carried on detailed investigations beyond some attempts at control.

Didymella applanata has been reported from England by Harris (17), Nattrass (26), Beaumont and Hodson (2), and Wormald (51). In Switzerland it has been reported by Osterwalder (30, 31), Müller-Thürgau and Osterwalder (25), Schellenberg (43), and Weiss (49). Osterwalder (30), in 1922, reports that "no satisfactory method of controlling the dieback of raspberry canes due to *Didymella applanata* has yet been devised. The results of experiments at Wädenswil with Bordeaux and lime-sulphur were all negative."

In Holland, spur blight was reported by Karthaus (21) in 1927. This writer found that the Didymella perithecia contained paraphyses only in immature fructifications. He suggests that this apparent absence of paraphyses has probably led to confusion between *Didymella applanata* and *Mycosphaerella rubina* (Pk.) Jacz. Karthaus observed a *Phoma* sp. on spur-blight lesions in August and also obtained it in culture. He thought the death of buds on infected canes was probably due to *D. applanata*,

though no actual connection was traced. *Coniothyrium fuckelii* (Sacc.) also was found closely associated with spur-blight perithecia.

In Germany, *Didymella applanata* is apparently widespread and causes a great deal of damage. It has been reported there by Rabbas (36), Theissen and Sydow (47), Pape (32, 33), Höstermann and Noack (18), Kirchner (23, p. 605), Schlodder (44), Rosenthal-Rötha (39), Schaffnit and Lüstner (42), and Burchard (4).

Burchard (4), in 1929, described the life history and symptoms of *Didymella applanata.* He described the ascospores as measuring 6 by 3 μ. The conidiospores which, he states, belong to a species of Phoma were described as being uniseptate, 4 to 5 μ in diameter, and discharged in long tendrils. He also states that pure cultures from the ascospores and pycnospores agreed in their mycelial characters. These spore forms do not agree in description or size with those of other investigators and the writer. The imperfect stage which he describes is certainly quite different from the species of Phoma usually described as belonging to *D. applanata.*

In Norway *Didymella applanata* has been reported by Gram and Rostrup (12, 13, 14), Gram and Thomsen (15), Ravn (37), and Jørstad (20).

In Denmark, it has been reported by Weber (48) and Jørgensen and Weber (19). The latter isolated *Didymella applanata* in pure culture and found the imperfect form to be a *Phoma* sp. These investigators agreed with Karthaus in considering that *D. applanata* and *Mycosphaerella rubina* are identical. They supposed that the apparent absence of paraphyses in the latter was apparently due to an oversight. *Coniothyrium fuckelii* was found in association with *D. applanata* on diseased canes.

They considered the most important source of infection to be the conidial (Phoma) stage of *D. applanata.* It was observed that many buds were killed during the winter months and numerous flowering and fruiting canes and shoots were weakened or withered prematurely. They found that the mycelium appeared to be confined to the cortex of the young shoots and that it was always intercellular.

ECONOMIC IMPORTANCE

In Europe, *Didymella applanata* is held responsible for a great deal of damage to raspberries. In England, Wormald (50) reports considerable injury to the canes. In Germany, Höstermann and Noack (18) state that the die-back of raspberry canes caused by *D. applanata* is constantly increasing in severity and a particularly virulent form of the disease resulting in the production of witches' brooms on the canes has been observed in some parts of Central Germany. Rabbas (36) reports that in 1921 over 70 per cent of the raspberry crop of an extensive plantation in Anhalt was

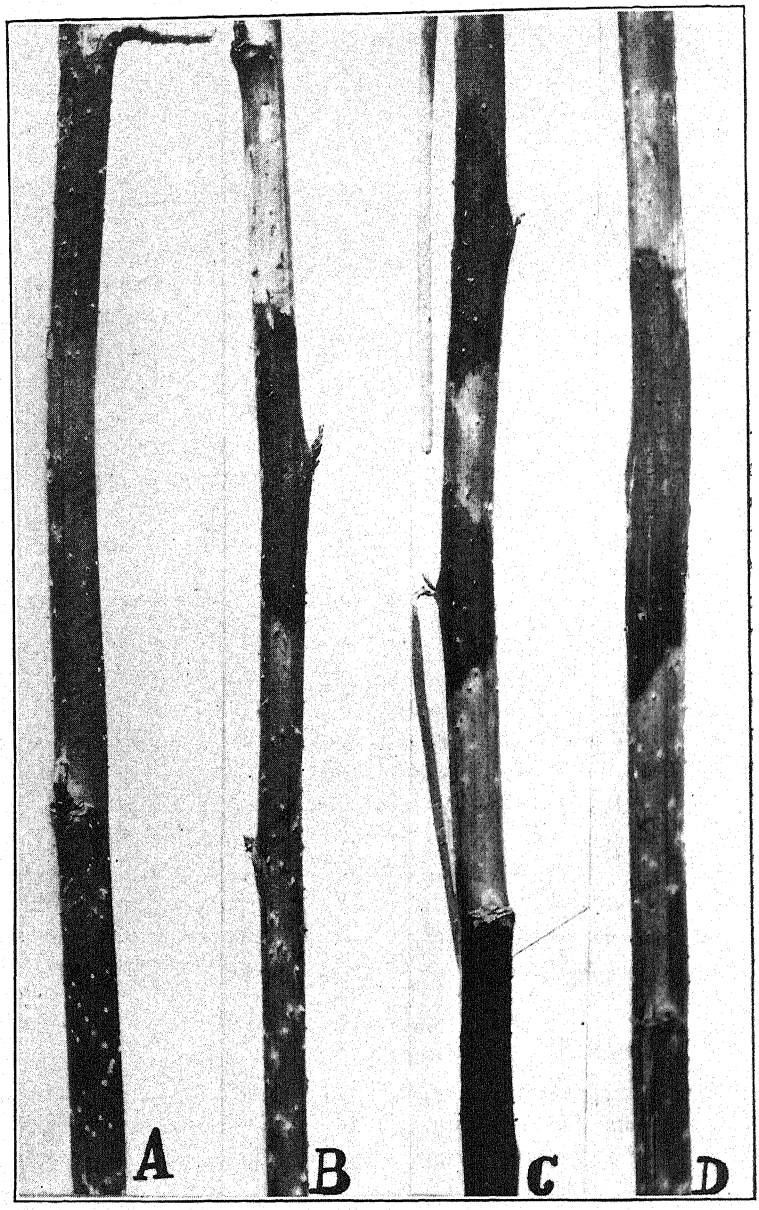


FIG. 1. A. Cuthbert cane free from spur blight in August. B, C, and D. Cuthbert canes infected with spur blight, showing the dark, discolored lesions surrounding the nodes.

destroyed by cane blight, due to *D. applanata.* In Holland, Karthaus (21) states that the death of the buds on the canes is probably due to *D. applanata,* though no actual connection was traced. In Denmark, Gram and Rostrup (13) state that raspberries were injuriously attacked by *D. applanata* on excessively nitrogenous soils in Fünen. Weber (48) reports that often the buds around which the infection occurs drop off. On the other hand, the buds may open but soon wither. It often happens that a badly infected raspberry plantation may appear perfectly sound when given a hasty examination in the early summer. There is every appearance of a good crop, but in ripening the fruits dry up and are useless.

Nicholls (28) reports that spur blight is one of the most destructive diseases in Tasmania.

Until recently spur blight has not been considered to be of economic importance in Ontario. However, it has become increasingly prevalent of late in the Niagara Peninsula, where it has been causing considerable damage, especially by way of bud injury. H. N. Racicot states in a letter to this laboratory that spur blight was very injurious in Quebec in 1926. In a number of plantations near Montreal, reduction in yield was 50 to 90 per cent.

SYMPTOMS

In Ontario, spur blight has been observed on young raspberry canes as early as June 25. Early infections are indicated by brown to dark or violet-brown discolored areas, the depth of color depending on the amount of surface bloom, which is not the same in all varieties. The discolored areas are to be found in most instances on the lower halves of young canes. They usually appear first at a node directly below the point of attachment of the leaves. Occasionally the discolored areas originate in other portions, such as the internode regions, on the buds, on the leaf petioles, and on the main veins of the leaves themselves.

The whole area surrounding the growing buds at infected nodal regions turns brown. (Fig. 1, B, C, and D.). The development of the buds in such regions is interfered with and by autumn they are either dwarfed to a considerable extent or, in many cases, by that time, they may have shriveled and dried up. (Fig. 2, A, 2.) When infected buds are not killed outright during the fall and winter, as frequently occurs (Fig. 3, B), they are so dwarfed and weakened that the following spring they are able to send forth only small, weak spurs which may come into leaf but which rarely reach the blossom stage (Fig. 2, D). The leaves on such diseased spurs are chlorotic and they exist in this condition for a few weeks and then usually die. In such cases the leaflets drop off, leaving the dried-up petiole attached to the cane. Often after the death of the main bud at the

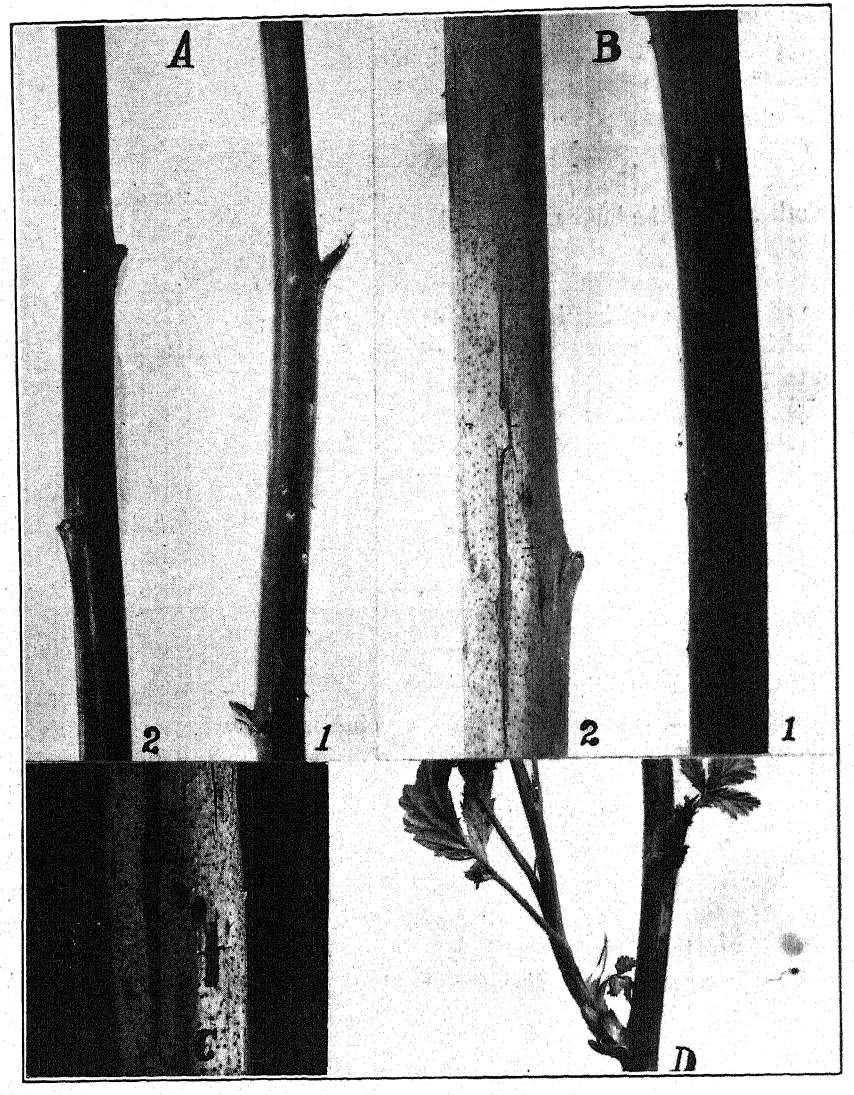


FIG. 2. A. (1) Normal Herbert cane in August. (2) Cane infected with spur blight, showing the dark brown epidermis, which is cracked, and the nodes where the buds became infected and dropped off. B. (1) Normal Herbert cane in May. (2) Cane infected with spur blight, showing the split cortex, the node where the infected bud has dropped off, and numerous perithecia of *Didymella applanata* on the gray surface of the cane. C. Portion of (2) B, magnified to show the abundance of perithecia on the surface and the splitting of the cortex. D. Fruiting cane of the Brighton variety in the latter part of May, showing an infected node from which is growing a typically dwarfed spur from an infected bud. Note the split cortex and the perithecia surrounding the node.

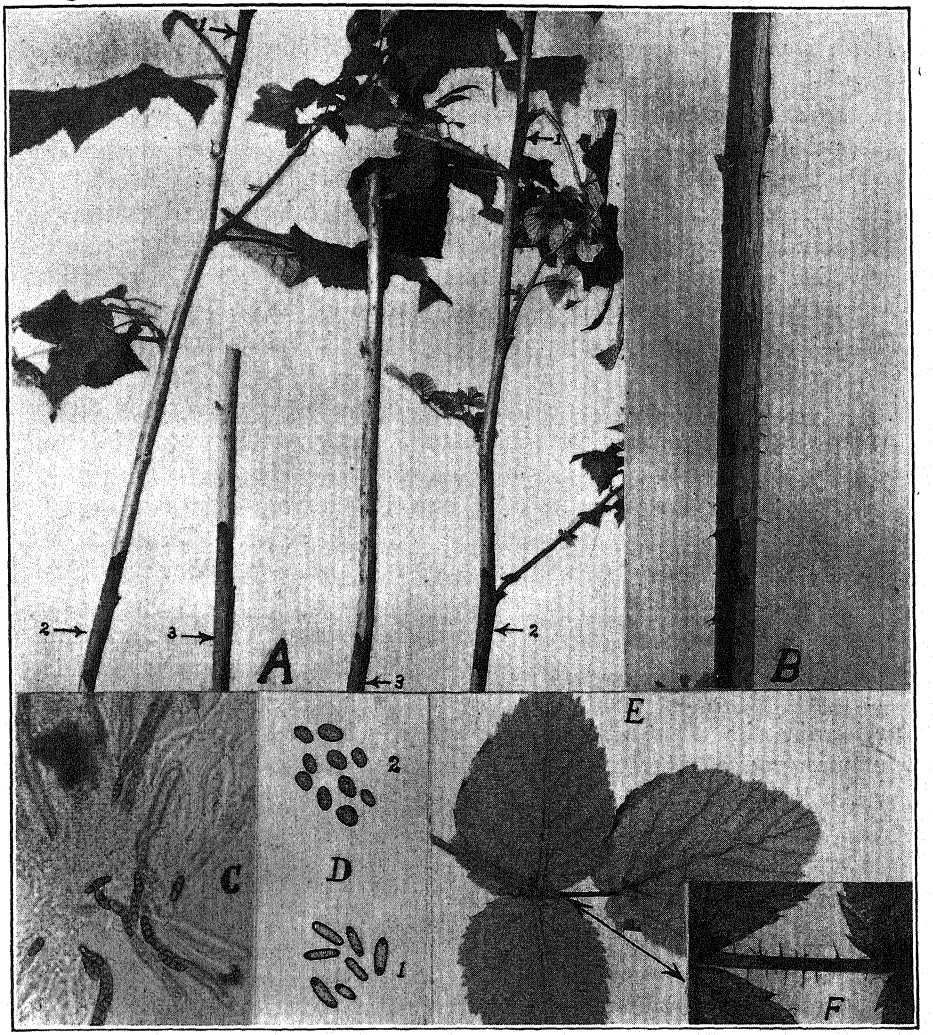


FIG. 3. A. Two Herbert canes inoculated in three places, 1, 2, and 3, one month previously with spur blight, showing the characteristic dark brown lesions. B. Fruiting cane of Cuthbert variety in May, showing a portion of the cane infected with spur blight. Within the infected area all the buds have been killed and the epidermis and cortex of the cane are cracked. C. Photomicrograph of *Leptosphaeria coniothyrium*, the perfect stage of the *Coniothyrium* sp., commonly associated with the spur-blight perithecia. × 310. D. (1) Pycnospores of *Phoma* sp., the pycnidial form of *Didymella applanata*. × 600. (2) Pycnospores of *Coniothyrium* sp., commonly found associated with the spur-blight perithecia. × 600. E. Erskine Park leaf inoculated with *Phoma* sp. Infection of the top leaflet has resulted in its death and the dark petiole shows that infection has traveled downward. F. Petiole of infected leaflet in E, magnified to show that infection proceeded down the petiole towards the other leaflets.

node, the tiny auxiliary bud immediately below develops a weak fruit spur which is never so large or healthy as the normal one. The final result of spur-blight infection is a cane with few or no fruit spurs for about the first 24 inches above the ground and, hence, with only the portions above this producing berries.

In many instances the infection of the nodal region can be traced to an infection of the petiole (Fig. 3, E and F), which has spread towards its base and invaded the tissue in the region of the bud. From the nodes the brownish discolorations spread to the internodes, and by the middle of August in many cases all of the lower portion of the cane is violet-brown (Fig. 2, A, 2). In some cases cane laterals are killed by the fungus early in their development.

If the diseased portions on the canes are carefully examined during August, especially after a wet period, small, scattered, brown pycnidia will be observed partially concealed by the discolored epidermis.

Invariably by the latter part of the season and very often much earlier the cortex of diseased canes splits longitudinally (Figs. 1, D; and 2, B2, C and D), after which the canes dry out and become very brittle. About the middle of September the brown discolored areas begin to take on a grayish aspect and by the first of November are quite gray (Fig. 2, B2). Simultaneously with this color change, numerous black pustules appear, breaking through the grayish epidermis. These are chiefly immature Didymella perithecia with often a few Phoma pycnidia intermingled. These black pustules gradually become more prominent during the winter months and, by April, are conspicuous against the grayish background of the cane. (Fig. 2, B2 and C.)

OBJECTS OF THE INVESTIGATION

So little has been recorded concerning the habits of the spur-blight fungus that a wide field remained open for investigation. The objects of these investigations were to study (1) the life history, (2) cultural characteristics, (3) spore production and dissemination, (4) pathogenicity of the causal organism, (5) pathological histology of the host, and (6) control measures.

TAXONOMY

Spur blight in Ontario is caused by an ascomycetous fungus, which is referred to *Didymella applanata* (Niessl) Sacc. for the reasons discussed in the following paragraphs. This species was first described by Niessl (29) in 1875 as *Didymosphaeria applanata*. Saccardo (40) transferred it to Didymella in 1882. Apparently some difference of opinion has arisen regarding the true relationship of the genus Didymella. Lindau, in Engler and Prantl (24, p. 431), assigns Didymella to the order Sphaeriales and

family Mycosphaerellaceae, while Gäumann (11, p. 221), on the other hand, follows Petrak (35) in assigning it to the family Pseudosphaeriaceae of the Myriangiales.

In America, the spur-blight fungus usually has been referred to *Mycosphaerella rubina*. It was first described by Peck (34) in 1894 as *Sphaerella rubina* and later was transferred to Mycosphaerella by Jaczewski, according to Stevens (45, p. 172). Since that time the spur-blight organism has been known by this generic name throughout America. Evidence presented in this paper, however, supports the view that the causal organism has been incorrectly assigned.

During the course of the early investigations, examinations of perithecia from local specimens of spur blight disclosed what appeared to be paraphyses. Since the taxonomic position of the fungus depended on the presence or absence of paraphyses, this feature was carefully investigated. Sections of perithecia from specimens collected from various localities in Ontario definitely established the presence of paraphyses. Figure 4, D, is a photomicrograph of a section of a perithecium from local material. It shows the paraphyses extending beyond the tops of the asci.

Through the kindness of Dr. H. D. House, curator of the New York State Herbarium, specimens were procured of the collections of spur blight made at Menands in 1894 from which Peck described *Sphaerella rubina*. Figure 5, D, is a photomicrograph of a section of a perithecium from this material and reveals the same characteristic paraphyses. All specimens of spur blight collected from Ontario, as well as the original type specimen of *S. rubina*, appeared to agree perfectly with the description of *Didymella applanata*. Comparison of Ontario collections of spur blight with a specimen of *D. applanata* from Germany (Sydow, Myc. Germ. 585) revealed no essential difference.

Dr. Harris, of East Malling Station, England, kindly furnished us with a culture of *Didymella applanata* grown on carrot agar and also with a number of infected canes. The infected canes appeared quite similar to our own specimens commonly attributed to *Mycosphaerella rubina*. A transfer from the English culture of *D. applanata* and one from a Canadian culture of the spur-blight organism were grown for comparison on the same medium and under identical conditions. After 14 days each culture had a white margin and the same characteristic olivaceous center and the two could not be distinguished. Both cultures developed later the same imperfect form, namely, *Phoma* sp.

For comparison of host symptoms, 20 inoculations were made by inserting bits of mycelium and spores beneath the epidermis. Ten of these were made from the English culture of *Didymella applanata* and 10 from a cul-

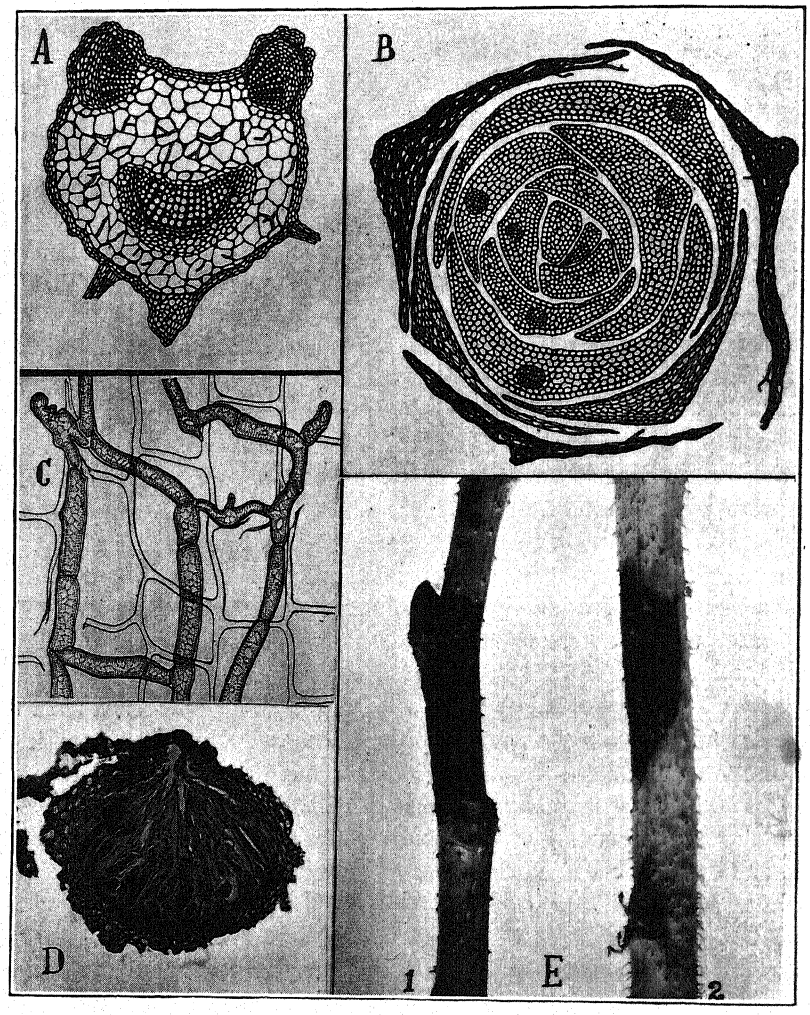


FIG. 4. A. Cross section of a petiole of a Cuthbert leaf infected with spur blight, showing mycelium throughout the cortical tissue. ×70. B. Cross section of a typical infected Herbert bud sectioned in the spring. The outer layers are killed completely and show the presence of mycelium. The layers beneath these show a partial killing of each unit. This type of infection frequently results in dwarfed fruit spurs. ×30. C. Long section of cortex of young cane, showing the habit of the mycelium in this tissue. ×750. D. Photomicrograph of a section of a perithecium of *Didymella applanata*, showing paraphyses, the blunt ends of which may be observed towards the ostiole. Section from a diseased cane collected at Port Dalhousie. ×150. E. (1) Cuthbert cane inoculated 22 days previously with a culture of *Didymella applanata* (local isolation), showing the typical brown lesion which developed. (2) Cuthbert cane inoculated 22 days previously with a culture of *Didymella applanata* (English culture) of the same age as the culture used in (1). Note the similarity of the brown lesions produced.

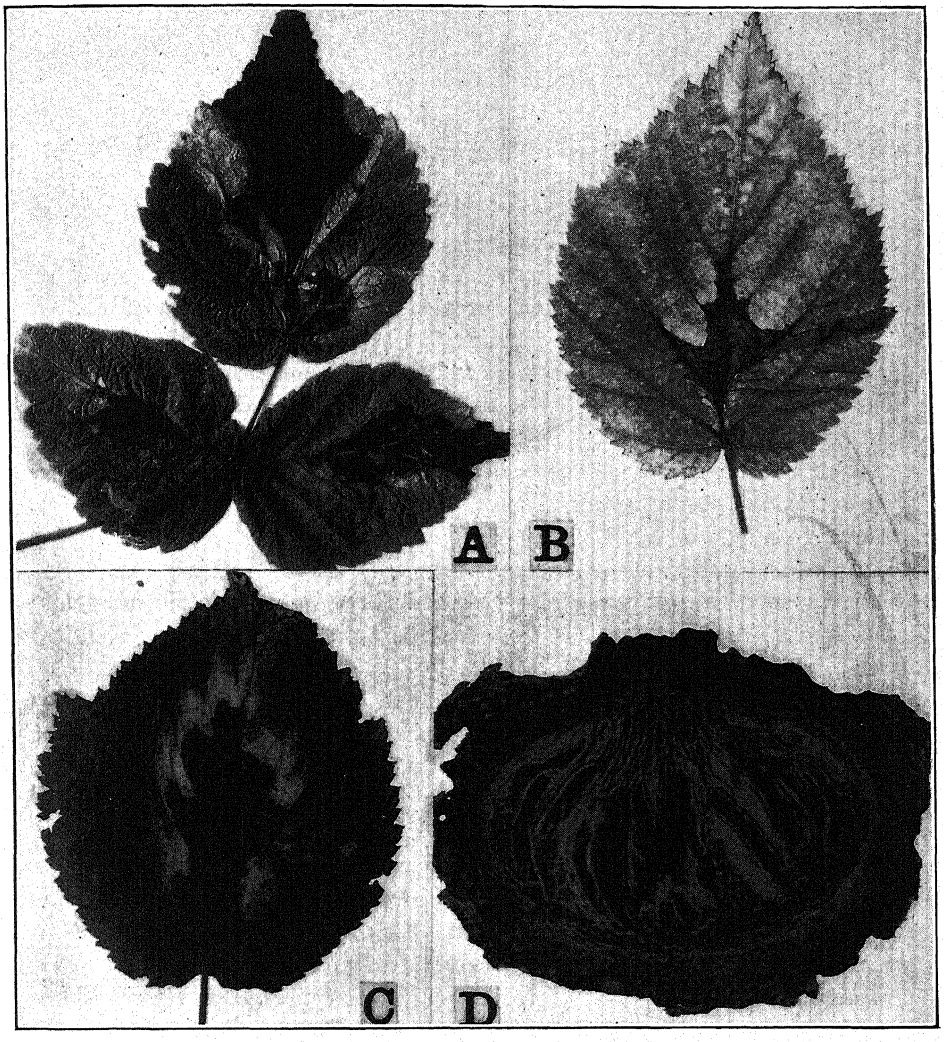


FIG. 5. A. Leaf of Herbert variety, showing natural infection by spur blight in each leaflet. Note the angular shape of the infected areas all of which originated on the main veins of the leaflets. B. Leaflet of Erskine Park variety which was injured and inoculated with *Coniothyrium* sp. Note the similarity of the necrotic area to that produced in C. C. Leaflet of Cuthbert variety which had been inoculated 24 days previously with a suspension of Phoma pycnospores. It will be observed that infection here also originated on the main vein. D. Photomicrograph of a section of a perithecium of *Didymella applanata*. This section was cut from material obtained from the New York State Museum and was part of the original type collection from which the spur-blight fungus was first described by Peck in 1894 as *Sphaerella rubina*. Paraphyses will be observed in this section extending beyond the tops of the asci. These were overlooked in the original description of *Mycosphaerella rubina*. ×340.

ture of our own spur-blight fungus. In all cases infection occurred, and those inoculated with our so-called *Mycosphaerella rubina* developed symptoms identical with those inoculated with *D. applanata.*

The result of this study strongly suggests that the causal organism of spur blight is the same on both continents and that the name *Mycosphaerella rubina,* which has been used exclusively for this organism in America, should be considered a synonym of *Didymella applanata.* The following is a summary of the evidence bearing on the identity of the two names.

1. Sections of perithecial material of the spur-blight fungus collected from various localities in Ontario revealed the presence of paraphyses.

2. Sections of perithecia belonging to the original material from which *Sphaerella rubina* was described by Peck proved the presence of the same characteristic paraphyses. These were apparently overlooked in the original description.

3. A culture of *Didymella applanata* from England developed the same characteristic Phoma pycnidia as our cultures of *Mycosphaerella rubina.* After 14 days, cultures of *D. applanata* from England and of *M. rubina* from Ontario were identical morphologically.

4. Inoculations of young canes with *D. applanata* from England and with *M. rubina* from Ontario made on the same date produced identical spur-blight symptoms.

5. A survey of the literature shows that, while both names have been applied to the causal organism of a similar disease, *M. rubina* has been limited in distribution to North America, while *D. applanata* has been reported from Europe and elsewhere but never from America.

Successful inoculations of *Didymella applanata* have been carried out on young raspberry canes by using ascospore suspensions. The ascospores mature in May and June in Ontario and evidence points to the fact that they furnish the chief primary inoculum for the disease. In addition to possessing a perithecial stage, it has been definitely established that this fungus develops an imperfect stage of the Phoma type. Young canes which were inoculated with ascospores from cultures of *D. applanata* developed Phoma pycnidia during the summer on the resultant lesions. During the following winter the fungus completed its life cycle by developing Didymella perithecia on the same lesions, which during the summer had developed Phoma pycnidia. Monascospore cultures of *D. applanata* always develop Phoma pycnidia and the ability of these pycnospores to produce infection has been repeatedly demonstrated by inoculation. This *Phoma* sp., which matures its spores in July and August, is responsible for secondary infections appearing late in the summer. These are frequently quite marked during a wet season.

LIFE HISTORY STUDIES

Investigations concerning the life history of the fungus were begun with the hope of obtaining information concerning (1) the characters of the perfect stage, especially a description of the perithecia, asci, and ascospores, and (2) the identity and description of the imperfect stage.

Canes infected with spur blight were collected from the Vineland, Port Dalhousie, St. Catharines, and Georgian Bay districts. Material from these localities was collected and examined for three successive summers commencing in 1927. Individual perithecia were macerated and examined in an unstained condition or were stained with a drop of alcoholic eosin, allowed to run under the cover slip. Representative samples of material also were embedded in paraffin, sectioned 5 and 10 microns in thickness, and mounted permanently. For staining sections, Delafield's haematoxylin and eosin were employed throughout the early part of the work and, later, Pianese 3b was found to be very satisfactory.

Perithecial stage.—Didymella applanata was observed to be constantly associated with the infected material. The perithecia often disclose a conspicuous circular ostiolum which is in the center and of lighter color than the surrounding area. When the perithecia are crushed under a cover slip the asci emerge in clusters united at the base, like bunches of bananas. These can be separated only with difficulty. Mature spores may be found in abundance by May 15, in Ontario. During the month of April the perithecia contain asci which are for the most part without mature spores, though mature ascospores were found as early as April 9. The perithecia and ascospores examined by the writer correspond in detail with Saccardo's (40) description of *D. applanata* (Niessl) Sacc., of which the following is a translation:

Didymella applanata (Niessl) Sacc. *Didymosphaeria applanata* Niessl, Neue Kernp., p. 129. Perithecia commonly gregarious, sometimes forming extended patches, submembranous, obscurely papillate, subglobose or depressed, at first covered by the epidermis, becoming superficial when the epidermis falls away, black; asci cylindrical or cylindrical-clavate, subsessile, 60–70 μ long × 10–12 μ wide; spores eight, biseriate, rarely uniseriate, obovate to oblong, uniseptate, constricted at the middle, upper cell larger than the lower, hyaline, 16 × 5.6 μ; paraphyses filiform, extending beyond the tops of the asci, simple. Habitat—on canes of dying *Rubus idaeus*, Shrewsbury, England (Plowright).

In table 1 measurements of perithecia, asci, and ascospores of *Didymella applanata* are given.

TABLE 1.—*Summary of the measurements of fifty perithecia, asci, and ascospores of Didymella applanata*

Range in microns	Mean size in microns
Perithecia (diameter.)	
165–220	195
Asci	
60 – 130.1 × 10 – 13.2	76.8 × 11.0
Ascospores	
14 – 20.2 × 5.5 – 9.1	16.5 × 9.1

ISOLATIONS FROM THE PERITHECIAL STAGE

Using material from the same scattered localities, indicated in the last section, a large number of isolations were made from the perithecial stage of *Didymella applanata.* Ascospore suspensions were made by macerating in sterile water from one to many perithecia obtained either from the same or from several infected canes. Cultures were grown on 2 per cent potato-dextrose agar and, where monosporous cultures were isolated, the method described by Keitt (22) was utilized.

In all, 156 isolations were made from perithecia. Of these, 68 were monascospore cultures. One hundred and fifty-three of these cultures developed a fruiting pycnidium of the Phoma type. Three cultures developed Coniothyrium pycnidia, but in no case were these derived from a monascospore culture. They resulted from pouring plates of what were supposed to be macerations of Didymella perithecia. It will be shown later that this *Coniothyrium* sp. belongs genetically to a fungus distinct from *Didymella applanata.*

Pycnidial stage.—In Europe a species of Phoma has been reported to be the imperfect stage of the spur-blight fungus *Didymella applanata* (21, 43). In America, however, spur blight has been attributed to *Mycosphaerella rubina* and the imperfect stage has been attributed both to *Phoma* sp. and to *Coniothyrium* sp. (45). No worker has completed the life cycle of the fungus on artificially infected canes and it was with this object in view that the writer undertook the following experiments.

Five Herbert and 3 Count plants were inoculated on July 16, 1928, with cultures originating from ascospores of *Didymella applanata.* On the same date 2 Adams 87 and 2 Erskine Park plants were inoculated with cultures of the *Coniothyrium* sp.

All canes developed typical brown spur-blight lesions. By September 15 all those canes inoculated with *Didymella applanata* disclosed the pres-

ence of Phoma pycnidia on the lesions produced. On the same date all canes inoculated with Coniothyrium cultures revealed the presence of Coniothyrium pycnidia on their lesions. During the winter months the brown discolored areas on the canes inoculated with *Phoma* sp. turned gray, and black perithecia became gradually more prominent. On examination these yielded only immature asci. On April 16 the writer was able to detect mature ascospores of *Didymella applanata* on a Herbert cane. One month later mature perithecia containing ascospores were found in abundance.

On the other hand, the canes inoculated with *Coniothyrium* sp. remained the same brown color during the winter months. Examination on various dates during the winter yielded Coniothyrium pycnidia only. On April 16, when the canes inoculated with Phoma cultures disclosed mature ascospores belonging to *Didymella applanata,* these canes still disclosed only Coniothyrium pycnidia with no signs of any change.

Thus the formation of perithecia belonging to *Didymella applanata* where Phoma pycnidia had developed during the previous summer as the result of inoculations with ascospore cultures of *Didymella applanata* definitely proved the genetic connection of *Phoma* sp. to *Didymella applanata.* The negative evidence obtained with *Coniothyrium* sp. under parallel conditions strongly suggested that it did not belong genetically to *Didymella applanata.* It remained, however, for the subsequent summer's work to establish the true genetic connection of the *Coniothyrium* sp.

A number of diseased raspberry canes were sent to the laboratory, examined by the writer on August 19, 1929, and found to contain along with spur-blight perithecia, mature perithecia belonging to *Leptosphaeria coniothyrium* (Fcl.) Sacc. Monascospore cultures of this fungus were made and these developed pycnidia 35 days later that belonged to the same species of Coniothyrium which has been suggested as belonging to the spur-blight fungus. Ten inoculations were then made on 10 Cuthbert plants. Sixteen days later examination of these canes revealed brown lesions similar in appearance to those caused by the *Coniothyrium* sp. which had been isolated in close association with the spur-blight perithecia. Close examination of these lesions disclosed the presence of Coniothyrium pycnidia similar in every way to those associated with spur-blight perithecia.

Summing up the foregoing evidence, we find definite proof of the genetic connection of *Phoma* sp. and *Didymella applanata;* also proof that the *Coniothyrium* sp. commonly found associated with spur-blight perithecia is the imperfect stage of *Leptosphaeria coniothyrium.*

Since the above evidence was obtained, numerous examinations have been made in the spring of canes which had been inoculated with *Phoma* sp. the previous summer, and in almost every case perithecia belonging to *Didymella applanata* were observed.

The following is a description of the Phoma stage of *Didymella applanata* as obtained from different host varieties.

Pycnidia separate, smooth; pycnospores borne singly, unappendaged; pycnidia free in the substratum, not beaked and opening by an ostiole; somewhat sunken in the substratum, at first covered, becoming erumpent at maturity; conidiophores simple; spores hyaline to light green in color, mostly two-guttulate and varying in shape from elliptical to oval.

Isolations from the pycnidial stage.—Monosporous cultures were made of Phoma pycnospores, employing the same methods as were used for the ascospores. These were made both from Phoma pycnidia in cultures which originated from ascospores and from pycnidia which developed on infected canes during the latter part of July and in August. In every case, cultures developed which were similar in every respect to those developed from ascospores, and Phoma pycnidia usually developed in these cultures after variable lengths of time.

Table 2 below gives measurements of *Phoma* sp. pycnidia and spores growing on various substrata.

TABLE 2.—*Measurements of pycnidia and spores of Phoma sp., the imperfect stage of Didymella applanata*

Pycnidia			
No. measured	Source	Size range in microns	Mean size in microns
25	Naturally infected canes	147–268 × 105–231	208 × 187
25	2 per cent potato-dextrose agar	114–170 × 80–144	143 × 118
Pycnospores			
60	Naturally infected canes	5.0–11.2 × 1.75–3.8	7.1 × 2.9
50	Naturally infected leaves	5.3– 8.0 × 3.0–3.8	6.6 × 3.3
50	2 per cent potato-dextrose agar	3.5– 8.4 × 1.5–3.6	5.5 × 2.3

The measurements in table 2 indicate a marked variability in the size of pycnidia and pycnospores that have developed under different conditions. Neither pycnidia nor pycnospores of the fungus became so large when grown on artificial media as when developed on the host, itself. Pycnospores on the leaves were also observed to be consistently smaller than on the canes proper.

CULTURAL STUDIES

Since the Phoma stage is the actively parasitic one throughout most of the growing season, its growth reaction in pure culture to various temperatures and to different culture media was studied. The following nutrient solutions in two per cent agar were used: Potato dextrose, prune, oatmeal, malt extract, raspberry decoction, and Pfeffer's and Duggar's synthetic media. Cultures were grown at the following temperatures: 2–5° C.; 8–11° C.; 23–24° C.; 27–28° C.; 30–31° C.

The relative growth rates at the various temperatures on the different media are shown in figures 6 and 7, where the maximum diameter of the culture in each case has been plotted against the length of the growth period in days. Growth occurred through the unusually wide temperature range of 2–28° C. The lowest temperature (2–5° C.) was evidently below the optimum, while the highest temperature (27–28° C.) was above it. At the other two temperatures (8–11° C. and 23–24° C.) growth was rapid and well maintained, the increment being smaller but more consistently maintained at the lower one. It seems probable from these results that temperature will not prove a limiting factor to the development of the disease.

The results indicate also a marked adaptability on the part of the fungus to a wide range of nutrients. The growth response to the various media, as indicated by the types of growth which resulted, was more varied, however, than might be suggested by the graphs. The most luxuriant growth and the most abundant sporulation occurred on potato-dextrose agar. An abundance of pycnidia also formed on oatmeal agar. The color of the cultures varied considerably on the different media but for the most part it was a golden brown after one week's growth. After three weeks most cultures were gray or a brownish-gray. Zonation frequently occurred in the cultures and appeared to be more closely related to the medium than to the temperature, though the influence of light in this connection was not checked. At 27–28° C. and at 30–31° C., cultures were slimy in appearance on all media. Subsurface mycelial growth was substituted for the aerial mycelium which occurred at other temperatures.

Pycnospores of *Phoma* sp. were found to germinate in sterile distilled water in 18 hours at 23–24° C., and in 48 hours at 8–9° C.

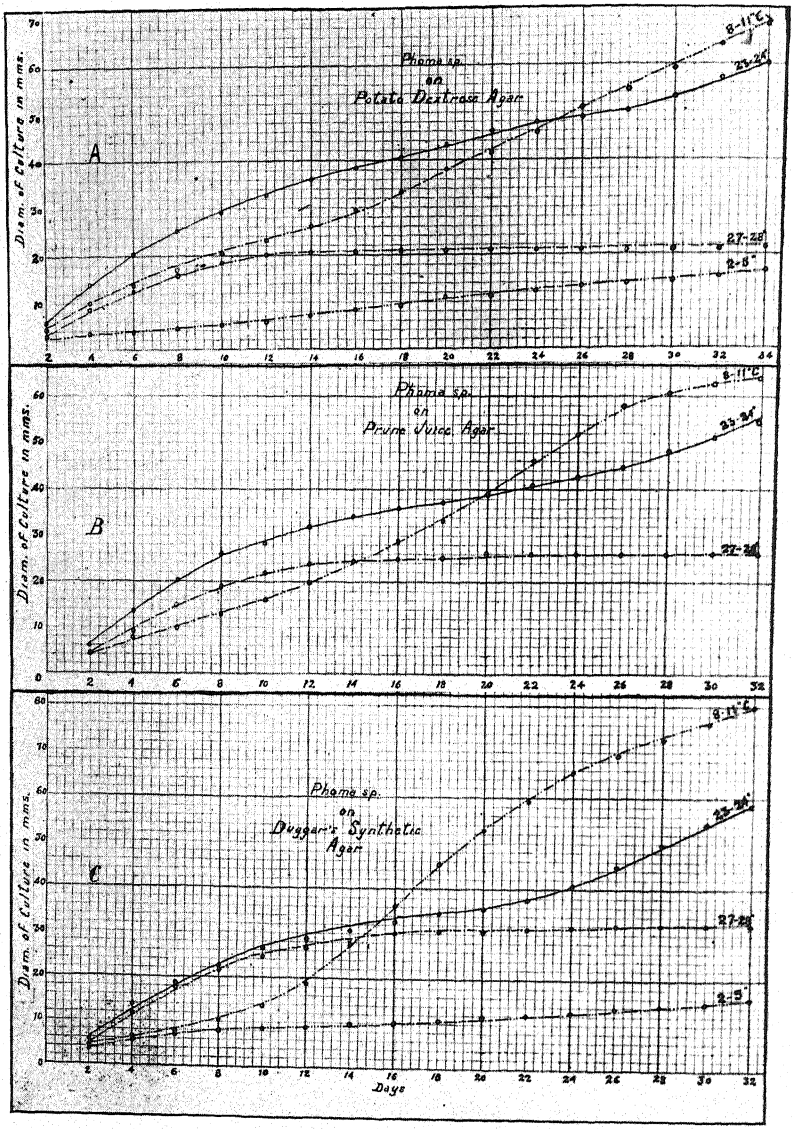


FIG. 6. Graphs showing growth rates of *Phoma* sp. grown, (A) on potato-dextrose agar at 2–5°, 8–11°, 23–24°, and 27–28°. (B) on prune-juice agar at 8–11°, 23–24°, and 27–28°. (C) on Duggar's synthetic agar at 2–5°, 8–11°, 23–24°, and 27–28°.

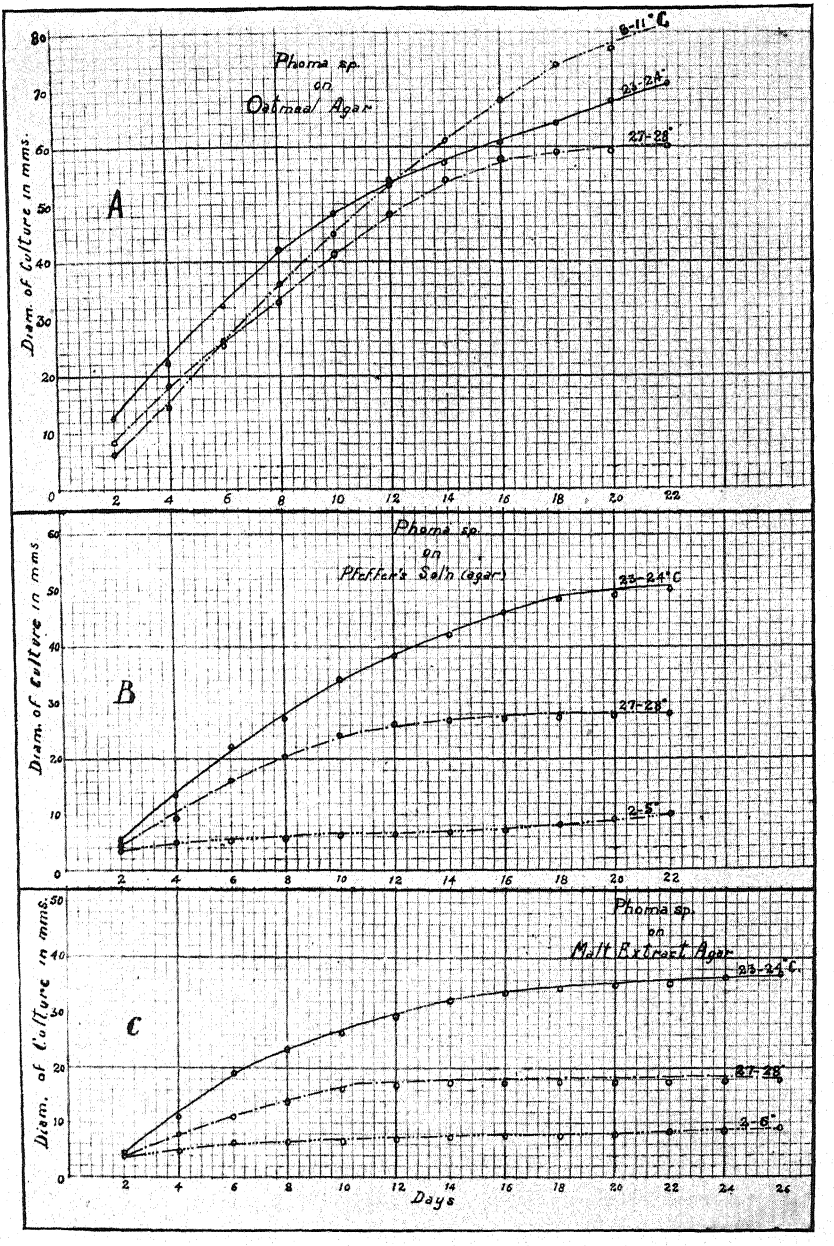


FIG. 7. Graphs showing growth rates of *Phoma* sp. grown, (A) on oatmeal agar at 8–11°, 23–24°, and 27–28°. (B) on Pfeffer's solution (agar) at 2–5°, 23–24°, and 27–28°. (C) on malt-extract agar at 2–5°, 23–24°, and 27–28°.

It would seem from the cultural studies conducted on various media that the fungus is able to thrive in the presence of starch or dextrose or both. The fact that the fungus thrives throughout a wide temperature range and on a wide range of nutrients may well account in part for its universal distribution.

PRODUCTION AND DISSEMINATION OF SPORES

Ascospores.—Since ascospores appear to be the important inoculum for initiating primary infection under Ontario conditions, an attempt was made to determine the time of their production and the conditions under which they are disseminated.

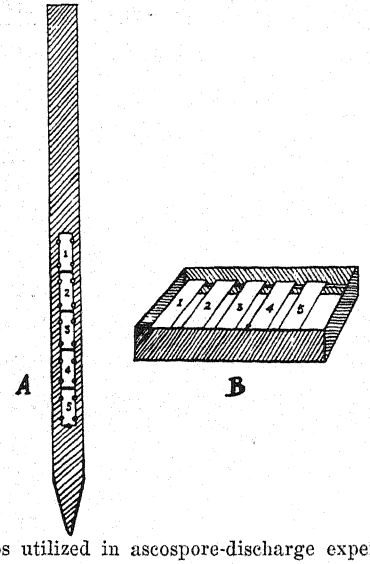


FIG. 8. Spore traps utilized in ascospore-discharge experiments. A. An ordinary lath on which were retained by tacks the vaselined slides. B. Rectangle inside of which five vaselined slides rested on a projection. The perithecial material was placed below the slides.

To study ascospore discharge, simple spore traps of two kinds were used. These are illustrated in figure 8, which gives the essentials of their construction. The first type consisted of an ordinary lath, on the side of which five glass slides lightly smeared with vaseline were held in position by means of tacks. When renewed after a shower the vaselined slides were pushed from beneath the tacks and fresh ones were put in their places. Five of these traps were stationed in different parts of a diseased plantation at Port Dalhousie. In all cases the pointed end of the lath was pushed into the ground alongside an infected cane and the lath was then tied to the cane in such a way that the vaselined surfaces of the slides remained about $\frac{1}{8}$ to $\frac{1}{4}$ inch distant from the perithecia on the diseased lesions.

The other type of spore trap consisted of a shallow open rectangle, 10 inches long by 3 inches wide. This rectangle was laid down flat and five glass slides were placed inside resting on tiny shoulders which ran lengthwise inside the rectangle. Infected canes, showing numerous perithecia, were then placed underneath the glass slides in such a position that they remained on the ground and $\frac{1}{8}$ to $\frac{1}{4}$ inch below the vaselined surfaces of the slides. Five traps of this type were stationed alongside the laboratory greenhouse and careful records of the spore discharge were made every few days. These traps were isolated from any plantation infected with spur blight in order to obtain an idea of what happens when growers cut infected canes in the autumn or winter and allow them to remain in heaps instead of burning them. More complete results were obtained from the traps stationed near the laboratory than from those in the plantation and the former are plotted in figure 9.

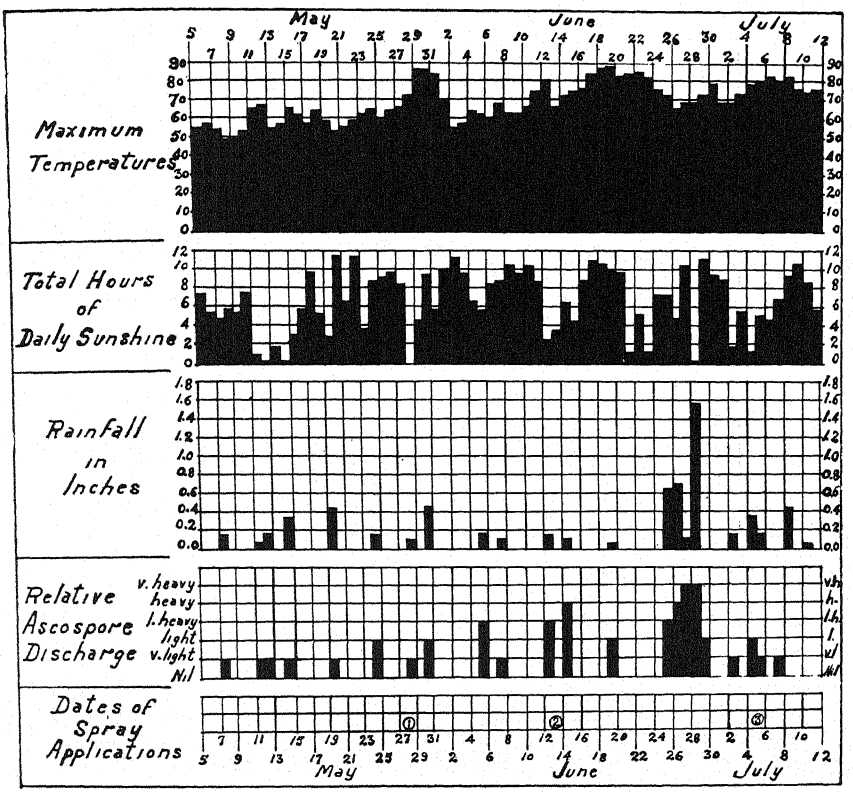


FIG. 9. Graph showing the relation of maximum temperatures, sunshine, and rainfall to the period and amount of ascospore discharge, as well as the dates of spray application.

The vaselined slides were examined as soon as possible after every rain and replaced by fresh ones. The results of these examinations are recorded below in table 3. In order to represent the results graphically in comparison with meteorological data, frequency classes were arbitrarily designated in the following way: Up to 2 spores per low-power field of the microscope (16 mm. objective and 10X eyepiece) were designated as "very light"; 3 to 5 spores, "light"; 6 to 10 spores, "light heavy"; 11 to 25 spores, "heavy," and 26 spores and up, "very heavy."

TABLE 3.—*Number of ascospores of Didymella applanata on exposed slides*

Date of rainfall	No. of slides examined	No. of fields counted	Average spore frequency	Location of trap
May 7/29	20	40	0.8	Lab. lawn
" 7	20	40	1.1	Plant"
" 11	20	40	1.4	Lab. lawn
" 12	20	60	1.0	Lab. lawn
" 12	20	40	1.8	Plant"
" 14	20	60	2.1	Lab. lawn
" 14	18	36	3.2	Plant"
" 19	20	50	2.0	Lab. lawn
" 19	18	36	3.6	Plant"
" 24	20	50	4.8	Lab. lawn
" 24	18	36	3.9	Plant"
" 28	24	48	2.6	Lab. lawn
" 30	24	48	5.6	Lab. lawn
" 30	18	36	8.6	Plant"
June 5	24	48	8.2	Lab. lawn
" 7	24	48	1.6	Lab. lawn
" 7	18	36	6.2	Plant"
" 12	18	36	8.8	Lab. lawn
" 14	18	36	19.8	Lab. lawn
" 14	16	32	22.6	Plant"
" 19	18	36	6.9	Lab. lawn
" 25	18	36	9.2	Lab. lawn
" 25	16	32	12.8	Plant"
" 26	18	36	18.1	Lab. lawn
" 26	16	32	21.1	Plant"
" 27	18	36	41.6	Lab. lawn
" 28	16	32	63.0	Lab. lawn
" 28	16	32	82.5	Plant"
July 2	16	32	3.2	Lab. lawn
" 2	16	32	1.7	Plant"
" 4	20	40	4.2	Lab. lawn
" 5	20	40	2.2	Lab. lawn
" 5	20	40	2.8	Plant"
" 7	20	20	1.3	Lab. lawn
" 7	20	40	1.7	Plant"

Standard meteorological records were available at the laboratory during the period of experimentation and comparisons of the results with the various meteorological data were made. Figure 9 shows the relation of various environmental factors to ascospore discharge and indicates the time at which the spray applications, which will be discussed later, were made.

Judging from the spores caught on the exposed slides, ascospore discharge began on a limited scale early in May, rose gradually to a maximum which was maintained throughout the latter half of June, and dropped very sharply to relatively insignificant amounts at the beginning of July.

The one environmental factor which appears from figure 9 to be definitely correlated with ascospore discharge is moisture. It seems safe to conclude that active discharge will occur only when saturated conditions obtain.

No single environmental factor and no obvious combination of them seem to account for the curve of ascospore discharge. In the early part of the season the limiting factor probably is maturity of the ascospores, and it seems likely that temperature and moisture most significantly influence maturity. After the bulk of the spores are mature, moisture in its influence on spore discharge probably assumes paramount importance.

Pycnospores.—The date of the first seasonal appearance of the Phoma stage is quite variable, apparently depending chiefly on moisture. In a wet season, such as the summer of 1928, it appeared earlier and was also much more abundant than in a dry season, such as the midsummer of 1929. It also has been observed on numerous occasions that it is much more abundant on infected canes in wide rows than in plantations where the rows are narrow or of the hill arrangement. This is probably again a moisture relation.

Pycnidia continue to appear on these lesions during the subsequent winter and spring. The Phoma pycnidia cannot be distinguished macroscopically from the Didymella perithecia and are very often intermingled with them. During the spring rains the pycnidia are frequently disseminated simultaneously with the ascospores. On the vaselined slides in the spore traps they occasionally appeared in considerable numbers. Such being the case, they must be considered as playing a small part at least in primary infection in the spring. That they are capable of causing infection has, of course, been proved many times. (See Infection Studies.)

INFECTION CAPABILITIES OF D. APPLANATA AND PHOMA SP.

On canes and buds.—Various infection experiments were conducted with a view to studying the pathogenicity of the spur-blight fungus. Since it is generally supposed that spur-blight in the spring is initiated by asco-

spores, attempts were made to prove their pathogenicity. Experiments were also conducted to ascertain what portions of the plant are susceptible to infection.

A summary of the results of the ascospore-inoculation experiments are presented below in table 4. Ascospore suspensions were used as inoculum except in those cases where it is indicated that bits of infective material were directly inserted into wounds. These experiments were all carried out shortly after a rain when the relative humidity was high and when the canes were wet.

In table 5 the results of the inoculation experiments on various portions of the plants are given. As usual, all inoculations were carried out on young canes except those on fruit spurs, which were made on fruiting canes. Checks were made for all infection experiments. These were always carried out in the same way as the inoculations except that no inoculum was applied. Inoculations of the fruit spurs reported in table 5 were made by inserting a bit of mycelium and spores beneath the epidermis. These were

TABLE 4.—*Summary of the results of inoculations of young raspberry canes with suspensions of ascospores of Didymella applanata*

Variety	Date of inoculation	Method of inoculation[a]	No. of canes inoculated	Results 2 weeks later
Herbert	June 11/28	No. 1	10	A definite spur-blight lesion appeared at every inoculation point
Cuthbert	June 11/28	No. 1	10	"
Herbert	June 20/28	No. 2	10	Nine canes showed one or more brown lesions
Cuthbert	June 20/28	No. 2	10	Eight canes showed one or more brown lesions
Herbert	June 25/28	No. 3	10	Every inoculation showed definite spur-blight symptoms
Cuthbert	May 31/29	No. 2	15	Twelve canes showed one or more brown lesions

[a] Methods of inoculation:

No. 1: Each cane was wounded to the cortex at a node with a flamed scalpel, in two places. Into these wounds ascospore suspensions were painted with a camel's-hair brush.

No. 2: Ascospore suspensions were atomized on the surface of apparently uninjured canes, chiefly at the nodes.

No. 3: Bits of diseased canes covered with numerous perithecia were inserted into the cortex of healthy canes at a node. Two inoculations were made on each cane.

made about 2 inches behind the small branched pedicels bearing the fruit. The culture used was 32 days old and contained pycnidia. In the same manner as the fruit spurs the growing tips were inoculated about 3 inches behind the branched tip. This culture was 28 days old and also contained pycnidia. Bud inoculations in 1928 were made by inserting Phoma pycnidia between the folded leaves at the extreme tip. In 1929, bud inoculations were carried out by painting a suspension of Phoma spores on the surface of the buds with a camel's-hair brush. All inoculations of buds were made when the relative humidity was high a short time after a rain.

TABLE 5.—*Summary of the results of inoculations of various portions of raspberry plants to determine their susceptibility to Didymella applanata*

Portion of plant inoculated	Variety	Date of inoculation	No. of inoculations	Results
Fruit spurs	Herbert	June 30/28	One on each of ten spurs	Ten days later, definite lesions surrounded each inoculation point. Sixty days after, inoculated spurs showed lesions 2 inches in length
Growing tips	Herbert	July 21/28	One on each of ten tips	Two weeks later, eight inoculated tips were dead. Penetration through the entire stem at that point was indicated. The remaining two showed large brown areas
Developing buds	Seneca	Aug. 7/28	50,—2 buds on each of 25 canes	In ten days the top third of all inoculated buds were brown. In 18 days the top halves were brown
Developing buds	Cuthbert	July 16/29	40,—2 buds on each of 20 canes	Three weeks later, 26 inoculated buds showed brown areas either at the tip or base

The results presented in table 4 indicate that spur-blight symptoms can be produced by artificially inoculating normal canes with ascospores of *Didymella applanata* (Fig. 3, A). The fact that apparently normal, sound canes will become infected after ascospores have been painted on their sur-

faces strongly suggests that the fungus is capable of penetrating non-wounded host tissue. From an examination of table 5 it will be observed that the same is true of apparently noninjured buds. This point is discussed more fully in connection with leaf-infection studies. Regarding the 1929 bud inoculations, it was observed that earliest infection appeared anywhere between the extreme tip and the base of the bud, though infection in an intermediate position occurred more rarely.

The results of inoculations of fruit spurs and the tips of young canes proved that these portions of the canes also are susceptible to infection. In the case of the growing tips the results were somewhat extreme, owing to a continued spell of wet weather subsequent to the inoculations. Nevertheless, the results indicated that under excessively moist conditions the fungus was able to penetrate the stem, including the xylem and pith tissue, thereby killing the portions above the point of infection. The checks for all of the above experiments were healthy at the time the observations were made, with the exception of three bud checks in 1928 and one in 1929. The Seneca plantation, in which the 1928 inoculations were made, was rather badly affected with the disease.

In addition to the infection experiments described in tables 4 and 5, in which *Phoma* sp. was the inoculum used, numerous inoculations were made using *Coniothyrium* sp. These were carried out because of the close association and supposed connection of this species with the spur-blight fungus. The conclusions arrived at as a result of these experiments were as follows: Initial symptoms as caused by inoculations with the spur-blight fungus and with *Coniothyrium* sp. are always identical. In many cases during the first summer the symptoms cannot be distinguished morphologically. During the second season, however, in most cases the canes inoculated with *Coniothyrium* sp. die outright, while those inoculated with *Phoma* sp. still remain alive. In some instances, however, canes inoculated with *Coniothyrium* sp. died during the season of inoculation.

On leaves: Methods.—The appearance in the field of Phoma pycnidia on necrotic lesions on leaves of canes infected with spur-blight suggested that these lesions were caused by the spur-blight fungus. Experiments were carried out, therefore, to determine whether the Phoma stage of *Didymella applanata* could attack leaves. The later experiments on leaf infection were carried on with a view to obtaining further evidence regarding penetration through sound host tissue.

In the early experiments the leaves were injured and inoculated with a Phoma culture. The injury was made by puncturing the midrib of a leaf with a sterile needle and a bit of mycelium with spores was inserted into the wound. Later, spore suspensions were used and were either atomized

TABLE 6.—*Summary of the results of inoculations of raspberry leaves with ascospores and pycnospores of Didymella applanata*

Variety	Date	No. of leaves inoculated	Method of inoculation	Inoculum used	No. of leaves developing symptoms
Viking	10/8/27	10	Leaves injured	Phoma (87 days old)	24 days later—7 leaves
Erskine Park	2/2/28	12	Spore suspension on non-injured leaves	Phoma (67 days old)	18 days later—9 leaves
Erskine Park	17/3/28	7	Leaves injured	Phoma (32 days old)	17 days later—7 leaves
Herbert	26/5/28	8	Leaves injured	Phoma (37 days old)	16 days later—8 leaves
Ohta	11/5/29	40	Spore suspension on non-injured leaves	Ascospores (perithecia macerations)	18 days later—11 leaves Three dead as a result of petiole infection
Herbert	21/5/29	40	Spore suspension (a) 20 noninjured leaves (b) 20 injured leaves	Ascospores (perithecia macerations)	19 days later—11 leaves (4 on noninjured and 6 on injured leaves)
Cuthbert	12/7/29	16	Spore suspension on non-injured leaves (a) 8 on upper surface (b) 8 on lower surface	Phoma (33 days old)	14 days later—5 leaves (3 inoculated on upper surfaces, 2 inoculated on lower surfaces)
Cuthbert	26/7/29	15	Spore suspension on non-injured leaves	Phoma (macerations of pycnidia from canes)	15 days later—8 leaves Three dead as a result of petiole infection
Cuthbert	1/8/29	8	Spore suspension on non-injured leaves (a) 4 on upper surface (b) 4 on lower surface	Phoma (42 days old)	15 days later—3 leaves (2 inoculated on upper surfaces, 1 inoculated on lower surface)
Cuthbert	11/8/29	11	Spore suspension on non-injured leaves	Phoma (52 days old)	16 days later—6 leaves

on the surface of the leaves or painted on with a camel's-hair brush. In these experiments, if the leaves were to be injured, needle punctures were made in various places. All inoculated plants in 1929 experiments were covered with bell jars for approximately 48 hours. Prior to inoculation, all leaves were washed with mercuric chloride (1:1000) and sterile water. Checks were made for all experiments in the customary way. In table 6 will be found a summary of the leaf-infection experiments.

The experiments outlined in table 6 indicate that leaves are susceptible to infection by *Didymella applanata* and that infection may occur either through wounds or through noninjured tissue. It was shown aleso that both noninjured buds and noninjured canes were artificially infected. This evidence, along with the fact that in badly infected plantations two thirds of all the buds on young canes are often infected, strongly suggests that *Didymella applanata* is not strictly a wound parasite or that it can take advantage of wounds of otherwise negligible proportions.

Examinations of raspberry leaves of the Cuthbert and Herbert varieties indicated that stomata are limited almost entirely to the under surfaces of the leaves. Only on two occasions were any stomata found on the upper surface and these were situated at the extreme margin of the leaf. In several of the experiments it will be observed that spores were painted on only the upper or under surface of the leaves. The percentage of infection in both cases was very nearly the same. Therefore the possibility of penetration through noninjured tissue being limited to stomatal or lenticular openings is very doubtful.

It also was observed that in nearly every instance when a noninjured leaf became infected some point on a vein proved to be the origin of the infection. The incubation period was about 2 weeks and infection became apparent when a tiny spherical brown area appeared somewhere on a vein. This gradually enlarged, extending more rapidly along the vein than in the mesophyll tissue (Fig. 5, A). After the infection became a week old or more, according to the weather conditions, the area of infection became more or less irregular in outline, commonly triangular in shape, with the widest portion towards the margin or tip of the leaf and the angle on the main vein of the leaflet pointing towards the petiole (Fig. 5, A). Almost invariably an intermediate yellowed portion was present between the dead brown tissue in the center of the infection area and the green healthy tissue beyond (Fig. 5, C).

It is also worthy of mention that raspberry leaves which were inoculated with *Coniothyrium* sp. were found to be susceptible to this fungus (Fig. 5, B). Heretofore infection by *Coniothyrium* sp. has supposedly been limited to the canes.

VARIETAL SUSCEPTIBILITY STUDIES

Infection experiments were conducted on as many raspberry varieties as possible in the hope of finding some resistant varieties. In addition, cross inoculations among a number of varieties were made to determine whether there was any difference in the pathogenicity of the cultures isolated from different sources.

Methods.—These investigations were conducted over a period of two years and were in most cases at least duplicated. The majority of the experiments were conducted in Mr. Corcoran's plantation at Port Dalhousie. Inoculations were performed by making a slight abrasion of the cortex with a flamed needle and inserting bits of mycelium and spores in the abrasion. Usually two or three inoculations were made on each cane. The cultures used varied in age from 8 to 33 days and in practically every case contained Phoma pycnidia.

When the results of these inoculations were compiled the varieties were classed as moderately resistant, susceptible, and highly susceptible. The degree of susceptibility was judged on the rate of development of artificially produced lesions and the size of the lesion two months after inoculation. For instance, the average length of nine lesions on Columbia canes two

TABLE 7.—*Inoculation experiments with the imperfect stage of Didymella applanata to determine varietal susceptibility*

Variety	No. of canes inoculated	Length of incubation period in days	Reaction
Adams 101	4	8	Susceptible
Adams 87	22	14 to 21	"
Brighton	10	15	Highly susceptible
Columbian	19	9 to 14	Moderately resistant
Cayuga	22	14 to 20	Susceptible
Cuthbert	22	14 to 19	"
Count	31	10 to 16	"
Herbert	46	7 to 10	Highly susceptible
Idaho	3	8	" "
King	22	14 to 20	Susceptible
Latham	10	16	"
Marlboro	22	15 to 22	"
Newman 23	12	22	Moderately resistant
Owasco	16	14	Susceptible
Ohta	6	14	"
Seneca	22	11 to 19	"
St. Regis	6	10	"
Viking	6	22	"
Erskine Park	6	15	Highly susceptible
Black Cap (Plum Farmer)	6	15	Moderately resistant
Wild Black Raspberry	4	16	" "
Rubus odoratus	4	18	Susceptible
Blackberry	4	No infection	Resistant

months after inoculation was 1⅛ inches, while the average length of the same number on Herbert canes inoculated at the same time was 5¼ inches. On comparing the prevalence of spur blight in plots of Columbia and Herbert varieties adjacent to each other, usually only a few infections were present on the Columbia canes, while the majority of Herbert canes would have at least one infection and in many cases the lower halves of the canes were completely brown. This evidence was always considered along with the inoculation results when deciding the susceptibility of a variety. In the majority of the experiments a number of isolations were made from the brown lesions which developed, in order to compare the fungus in the lesions with the original inoculum.

Discussion.—The checks for the above experiments were all normal one month after the inoculations were made, with the exception of three Herbert and one Viking. In these cases brown lesions appeared on portions of the cane other than around the points of inoculation, thereby indicating that natural infection had occurred.

The results, which are summarized in table 7, indicate that all the varieties of red raspberries which were inoculated were more or less susceptible. Newman 23 displayed some resistance, as did also Columbian, a purple variety. Black varieties appear to be moderately resistant. On the other hand, the Herbert, Idaho, Brighton, and Erskine Park varieties proved highly susceptible and in a wet season, such as the summer of 1928, few canes in plots of these varieties escaped infection.

In the cross-inoculation experiments, no constant difference in pathogenicity was observed. There were, of course, differences in the growth rates of lesions even on the same variety and plant, but these were not consistent enough to be of any differential value. Our results do not indicate therefore, the existence of any physiological specialization in *Didymella applanata.*

PATHOLOGICAL HISTOLOGY

As has already been shown, *Didymella applanata* attacks primarily the canes and buds of raspberries. A study of the pathological histology of these organs was accordingly made.

Methods.—Normal and diseased host tissues were fixed in medium chromacetic and embedded in paraffin for sectioning. The diseased material included all stages from the first tiny brown spots which develop in June to the gray canes with mature perithecia which had been infected about one year. Both naturally infected and artificially inoculated material was examined. For many purposes, sections of fresh material cut with the microtome or razor and stained in cotton blue were thoroughly satisfactory. Permanent mounts were stained variously. Delafield's haematoxylin, eosin,

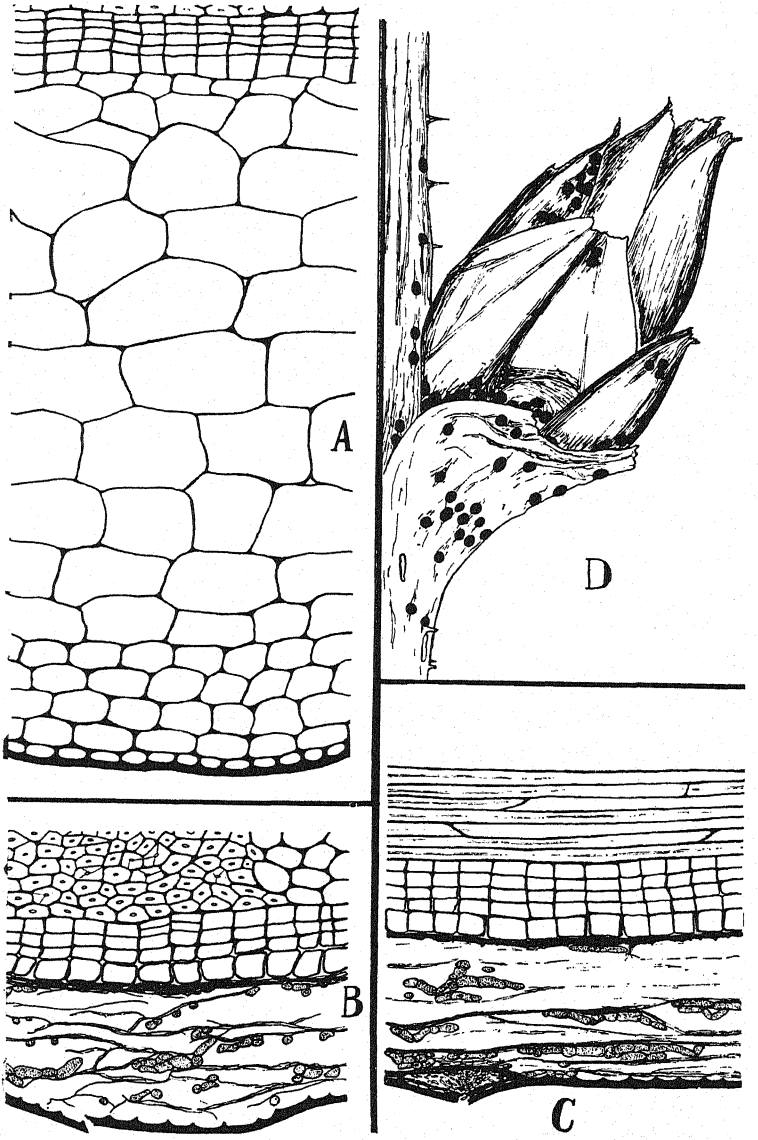


FIG. 10. A. Cross section of normal Herbert cane, showing normal amount of cortical tissue. ×600. B. Cross section of Herbert cane infected with spur blight, showing mycelium of *Didymella applanata* in the cortex which has been reduced to practically nothing. Note the amount of cortex in the diseased cane as compared with that in A. ×600. C. Longitudinal section of diseased Herbert cane, showing mycelium and a Phoma pycnidium beneath the epidermis. D. Drawing of naturally infected bud, showing perithecia of *Didymella applanata* on the surface of the bud scales. The bud was apparently killed by this fungus.

and Pianese 3b were those chiefly used. The latter proved quite satisfactory in most cases. Infected buds were treated in a similar manner. The histology of leaf petioles artificially inoculated with spore suspensions of Phoma were also studied from freehand sections.

Results. Cane histology.—In the normal current-season raspberry cane there are three well-defined cortical regions external to the cork (Fig. 10, A). The first region, which lies just beneath the epidermis, consists of from two to four layers of relatively small, thick-wall cells. The next region is composed of large, thin-wall cells and is usually eight to twelve layers in thickness. The last region, which composes the cork, consists of two or three layers of small thin-wall cells.

In all diseased specimens examined in which chocolate-color lesions appeared on the surface, all regions of the cortex except the cork were freely invaded by the mycelium of the fungus. The walls and their contents in all cases turned brown, presented a shriveled aspect, and appeared quite dead. The mycelium of the fungus in almost all cases was found to be intracellular, only a very few cases of it being observed intercellularly. Normally, the mycelium of the fungus appears to be limited to the cortical regions (Fig. 10, B and C). However, in sections of canes inoculated 10 months previously, mycelium was frequently observed in the phloem and xylem, but only when the cork tissues were injured and then only in the vicinity of the injury. In several instances of naturally infected canes mycelium was also observed beyond the cortical layers, but, on close examination, mechanical injury to the cork layers nearby was detected in every case. Under our conditions it appears that the cork forms an efficient barrier to the fungus so far as actual mycelial penetration is concerned. In cortical tissues the mycelium apparently travels directly through the cell walls (Fig. 4, C) and shortly afterwards the walls collapse and the cells die. In extending its boundaries up and down the cane the fungus invades the outside tissues first and later extends inward to the cork layers (Fig. 11, A). Microchemical tests of normal canes show a plentiful supply of starch in the cortical tissues in August. Similar tests of infected specimens at the same time show a complete absence of starch. The cork tissue does not become directly invaded by the mycelium of the fungus, but the few outer layers adjacent to invaded cortical tissue in most cases die and shrivel up (Fig. 10, B and C). Frequently all of the cork layers are browned. In many cases the phloem also is browned, when in close proximity to the mycelium, even though no penetration has occurred. In some instances in the phloem and medullary rays of infected canes there is an accumulation of starch greater than in normal ones.

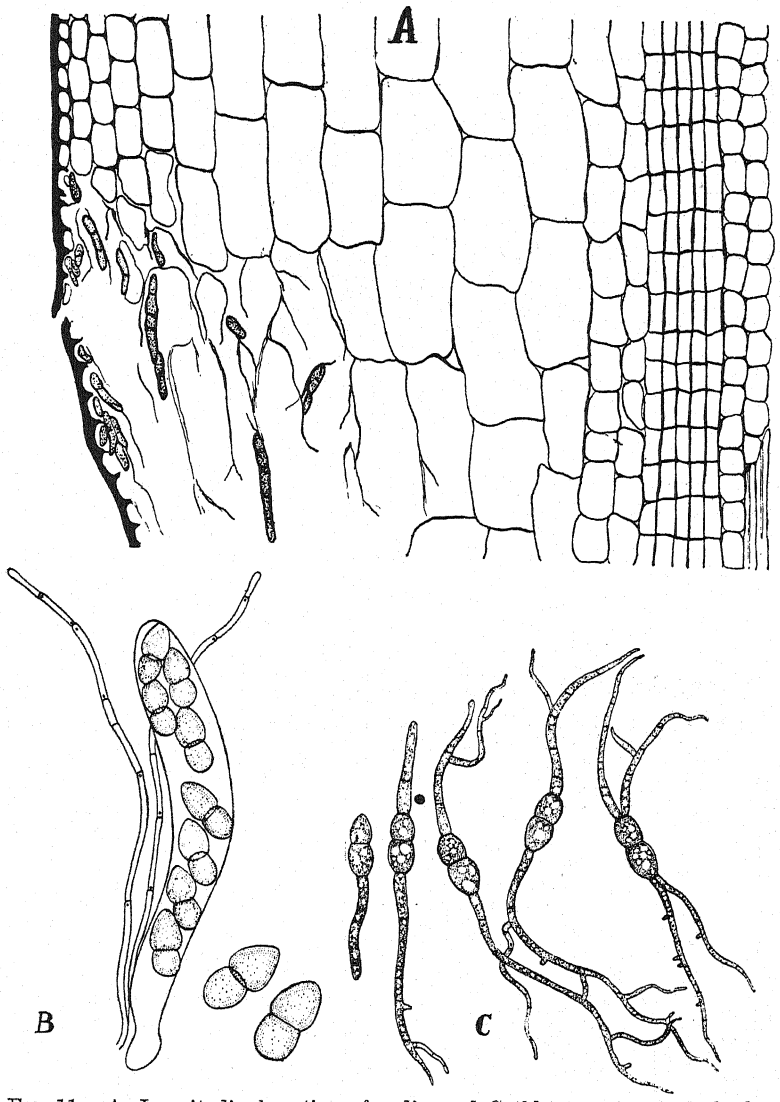


FIG. 11. A. Longitudinal section of a diseased Cuthbert cane cut at the boundary between brown and green tissue and showing the intracellular mycelium in the external layers of the cortex. ×600. B. Single ascus of *Didymella applanata*, showing the subbiseriate arrangement of the ascospores. The paraphyses which extend beyond the top of ascus have cross walls. ×800. C. Germinating ascospores of *Didymella applanata*, showing anastomosis of two germ tubes. ×500. (After 18 hours in sterile water at room temperature.)

Bud development and infection.—Rather an interesting point in connection with the bud development was observed regarding the small bud which is always present immediately below the large developing bud. In prepared sections of embedded material sections of both buds frequently appeared on the same slide. In some cases the tiny bud had apparently been killed by the fungus while the large one was not, and *vice versa*. In other cases both were killed. Observations during the subsequent summer yielded the following results. In most instances this small bud developed into a small leafy shoot with tiny leaves during the summer. This leafy shoot was located immediately below the large fruit spur. In many instances they were observed to die about the middle of July. In some cases, however, as, for example, when spur buds died, the small buds developed into comparatively large fruit spurs, but in most instances they were not so large as true fruit spurs.

Peculiar variations from the above were observed in several instances. Sometimes both spur buds and tiny buds developed to a large size, giving the cane a brushy appearance. Normally, however, the small buds developed into tiny shoots.

Close examinations of diseased buds in the spring in many cases disclosed fruiting bodies on the surface of the outer scales (Fig. 10, D). These often occurred in abundance towards the base of the scales. Examinations of these proved them to be chiefly perithecia belonging to *Didymella applanata* with a few Phoma pycnidia intermingled. Occasionally they were also observed inside the outer scale layer on the surface of the second layer. Examinations of buds infected only a short time previously showed that infection began in many cases at the extreme bud tip. Here only the tips of the outer scale layers protrude and might naturally be expected to become infected first.

Infected buds were almost invariably found to be considerably dwarfed. Comparative length measurements of 136, each, of normal and infected Cayuga buds, made on August 29, 1928, indicated an average dwarfing of 40 per cent. Similar measurements of 162, each, of normal and infected Cuthbert buds, made on August 28, 1928, indicated an average dwarfing of 27 per cent in that variety. This dwarfing which apparently resulted from spur-blight infection partially explains the weak fruit spurs which develop in the spring from infected buds (Fig. 2, D).

Bud histology.—Sections of buds which were infected during the summer and sectioned the following spring yielded most interesting results. Copious mycelium was found within the bud tissue. In the majority of infected buds only several of the outer layers were invaded by the fungus (Fig. 4, B). In all the layers which were invaded the tissue was badly

broken down and always presented a brown and shriveled aspect. The line of demarcation between affected and healthy tissue was as definite as in the case of diseased canes. The mycelium was found to be chiefly intracellular. The buds did not develop any protective layer or any visible barrier to mycelial spread. The mycelium apparently passed directly through the cell walls, after which the cells collapsed. Mycelium was not found in the vascular tissue of the bud but appeared always to be limited to the parenchyma. Often, however, the phloem was browned and the cells were shrunken, though mycelium was never observed in them. In a few exceptional cases mycelium was found in the central bud layers, killing the bud.

Buds sectioned in the autumn invariably disclosed less mycelial penetration than those sectioned the following spring. In numerous cases the tissue immediately below the bud was affected and brown, though sections of the bud tissue disclosed no mycelium. In such instances, however, the bud was nearly always considerably dwarfed. Examination of these buds in late summer showed a decided shrinking of the phloem cells with occasional browning. An examination of 200 naturally infected buds during the first week in August showed that infection had originated at the bud tip in 147 cases; in 18 infection had begun either at the base or at an intermediate position and in the remaining 35 the point of origin was uncertain.

Histologic reaction to the fungus.—In sections of leaf petioles which were made at various times mycelium of the fungus was observed to be very abundant throughout the cortical tissue (Fig. 4, A). The petiole itself was always much dwarfed and shrunken, due to the disintegration of the cortical regions. The mycelium was observed to be wholly intracellular and varied greatly in thickness in individual cells. On passing through a cell wall the mycelium on one side of the wall frequently appeared thicker than on the opposite side. Again no mycelium was found actually within the vascular tissue. Within the leaf tissue itself mycelium was found in the mesophyll.

From the results indicated in the section on histology it seems that under Ontario conditions *Didymella applanata* is essentially a parasite of cortical tissues. Observations indicate that under abnormal conditions, such as wounds in the cork, the fungus will penetrate the vascular tissues. Infection of buds frequently originates at the tip and extends down the bud scales during the summer and autumn, gradually killing the outer layers. The effect of the shriveling of the outer layers is to cause a separation of the layers at the bud tip, and consequently the inner succulent and tender tissue is left exposed. In this way buds infected with spur blight go into the winter in an exposed condition and are easily killed.

In many instances, infected buds that are not actually winter-killed are either dwarfed or weakened to such an extent that they are able to put forth only a small weak spur which bears fruit of no value. The killing of the tiny buds below the main spur buds appears also to be of some significance when the spur bud is killed, the result being that at that node no fruit spur is possible.

CONTROL MEASURES

Spraying experiments on the control of spur blight were begun on May 30, 1928, and were continued during the summer of 1929. During the first year a Bordeaux mixture was employed which consisted of 3 lbs. copper sulphate, 5 lbs. hydrated lime and 40 gals. water. To this was added 2 lbs. of laundry soap. The same mixture was used the second year except that 2 lbs. of whale-oil soap was substituted for the laundry soap as a spreader. Instead of the third spray in 1929 Bordeaux dust was applied with a blower, since the power sprayer was out of order.

First applications were made on May 30 and 28; second applications on June 12 and 14; and third ones on June 27 and July 6, respectively. A 40-gal. power sprayer was employed, and the spraying was carried out at a pressure of 250 lbs. with as fine a mist as possible, in order to eliminate mechanical injury as much as possible. Mr. Corcoran, of Port Dalhousie, kindly allowed the experiments to be conducted in his plantation both years. In 1928 one row of Herberts and three rows of Cuthberts were selected for spraying. Each row was 105 yards long and only one half of each row was sprayed, the other half being allowed to remain as a check.

Percentage control was calculated in two ways: First, on the basis of the number of diseased canes; and, second, on the total number of lesions in a group of canes selected at random.

TABLE 8.—*Summary of results of Bordeaux spraying experiments to control spur blight*

Variety	Number of sprays	Average percentage control for two years	
		By diseased canes	By lesions
Herbert	1	77.9 (one yr.)	92.4
	3	66.6 (one yr.)	79.2
Brighton	3	67.2	73.5
Cuthbert	1	70.3	85.9
	2	79.3	89.5
	3	86.1	97.9

The results of the spraying experiments are summarized in table 8. It will be seen that spur blight has been fairly sucessfully controlled by the use of a Bordeaux spray. The results were not always consistent, however, and the experiments must be continued over a longer period of time before definite conclusions can be safely drawn. The marked effectiveness of a single early application of Bordeaux mixture in 1928, when the disease was abundant, suggests that the timeliness of the application is all-important and that one spray, applied early enough to protect the plants against the heaviest of the ascospore discharge, may hold the disease satisfactorily in check that season. The advisability of additional applications will probably depend on the season and on local conditions.

While no appreciable spray injury has been observed in any of our plots, this is a matter which deserves further consideration in view of the fact that raspberries are notoriously susceptible to spray injury. While it seems likely that the lack of injury in our experiments was due to the strength of the Bordeaux mixture and the care with which it was applied, it is just possible that environmental factors peculiar to the district also exerted an influence. Until more data are collected, therefore, Bordeaux should be used cautiously on raspberries and in a more or less experimental way.

A summary of measures suggested for controlling spur blight in Ontario is as follows:

1. Apply a Bordeaux spray (3:5:40) to which has been added 2 lbs. whale-oil soap to young canes in May when they are 5 to 9 inches high. A second application may sometimes be warranted approximately 2 weeks later.

2. Do not allow the rows to become too deep. When rows are deep and close together there is less air drainage and moisture is retained longer and invariably more disease is observed in such plantations. The presence of weeds between the rows is likewise an important factor in increasing the humidity.

3. Avoid setting out plantations on sites which are poorly underdrained or surface-drained. Such plantations become an easy prey to a fungous disease like spur blight.

4. Procure nursery stock from disease-free plantations.

5. Avoid setting out plantations adjacent to patches of wild raspberries since spur blight is almost invariably present on the wild varieties.

SUMMARY

1. Spur blight is almost universal in its distribution, occurring in North America, Europe, and Australia.

2. Spur blight of raspberries is becoming more prevalent in the Niagara peninsula.

3. The history of the disease in America and Europe is outlined.

4. In Europe damage caused by spur blight is reported to be extensive in several countries, especially Germany and Switzerland. In America it appears to cause considerable damage in the United States and Canada.

5. In the summer the most striking symptoms of spur blight are purplish brown discolorations on the canes and similar lesions on the leaves and buds. Diseased canes turn light gray in color during the winter following infection. Minute black perithecia become increasingly conspicuous on the gray lesions during the winter and spring.

6. The disease is caused by *Didymella applanata* (Niessl) Sacc.

7. In America spur blight has been attributed to *Mycosphaerella rubina*. In Europe a disease having the same symptoms is considered to be caused by *Didymella applanata*. Mature perithecia of spur blight collected in Ontario and some from the original material from which *M. rubina* was described in 1894 by Peck were sectioned. The two were identical and paraphyses were present in both. Cultures and naturally infected specimens of *D. applanata* from England agreed morphologically with our own cultures and specimens. Inoculations and reisolations indicated that the two species were identical.

8. A description of the perithecial and pycnidial stages of *Didymella applanata* and the results of isolations from each stage are given. Perithecia belonging to *D. applanata* developed on canes inoculated with cultures originating from ascospores of the same species. These canes, prior to the formation of perithecia of *D. applanata* developed pycnidia belonging to a *Phoma* sp., which is the imperfect form. The Coniothyrium which is at times found associated with spur blight and which has been reported genetically associated with *D. applanata* was shown to be the imperfect stage of *Leptosphaeria coniothyrium* (Fcl.) Sacc.

9. The imperfect stage, *Phoma* sp., was cultured on eight different nutrient media. Growth rates of the fungus on the different media at various constant temperatures are given. The fungus can grow on artificial media at temperatures of 2° to 28° C.

10. Perithecia of *Didymella applanata* discharged their ascospores in 1929 from May 7 to July 7. A shower appeared to be necessary for ascospore discharge. Pycnospores of *Phoma* sp. were discharged at intervals along with the ascospores.

11. Infection resulted from the inoculation of both noninjured and injured canes with ascospore suspensions of *Didymella applanata*. Various portions of the plant, including fruit spurs, tips of canes, and developing

buds, proved to be susceptible to the fungus. Leaves were inoculated with ascospore suspensions of *D. applanata* and pycnospore suspensions of *Phoma* sp. Both noninjured and injured leaves developed typical symptoms as a result.

12. Twenty-two varieties of red and black raspberries were found to be more or less susceptible to spur blight.

13. All regions of the host cortex except the cork were found to be freely penetrated by the mycelium. The host produces no visible barrier to withstand invasion by the fungus in these portions. Perithecia of *Didymella applanata* were observed on the surface of bud scales in the spring. The direct invasion of bud tissue by the fungus was established. Several of the outer bud layers are those chiefly affected.

14. A 3:5:40 Bordeaux mixture, to which was added 2 lbs. of whale-oil soap, was employed in attempting to control spur blight. Over a period of 2 years an average control of 80.4 per cent was obtained with a single application.

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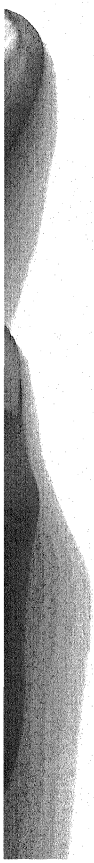
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THE LATE BLIGHT OF THE SUGAR BEET[1]

B. L. RICHARDS[2] AND C. M. TOMPKINS[3]

INTRODUCTION

In 1922 the senior writer (20) called attention to a disease of the sugar beet which appeared in an epidemic form in the beet fields of Utah during 1919 and 1921. At that time the trouble was designated as "late blight," and it was pointed out that, while *Phoma betae* (Oud.) Fr. was associated with a final and conspicuous root-rot stage of the disease, the initial phase of the trouble probably was nonparasitic in nature and, in its epidemic appearance, was closely associated with certain climatic conditions, intensified by such local factors as delayed irrigation, poor tilth, and low soil fertility. The disease referred to as late blight appeared again in a mild nonepidemic form in the sugar-beet fields of Utah in 1924, 1926, and 1929.

The purpose of this paper, while making no claim to completeness, is to characterize this peculiar disease of the sugar beet and to discuss some of the factors underlying its epidemiology in Utah.

SYMPTOMS OF LATE BLIGHT IN UTAH

As stated in an earlier publication (20), late blight makes its appearance in epidemic years as early as July 1 to 15 and continues its ravages throughout the remainder of the growing season. In seasons less favorable for blight development the initial symptoms may not develop until mid-August or as late as September or early October.

Late blight manifests itself by a sudden collapse of irregular areas of tissue of the leaf blade. These lesions may vary in size from mere pinhole spots (Fig. 1, A) to areas involving the entire tissue included between two main lateral veins (Fig. 1, B, C, D, E, F). Under severe conditions the entire tissue of the leaf blade, with the exception of the midrib, the large lateral veins, and the tissue immediately adjacent to these structures, becomes suddenly involved and, when dry, presents an appearance as if scorched by fire (Fig. 1, B, C, D, E). When conditions are especially favorable for disease development the large lateral veins with portions of the midrib may be killed, which results in complete collapse of the leaf

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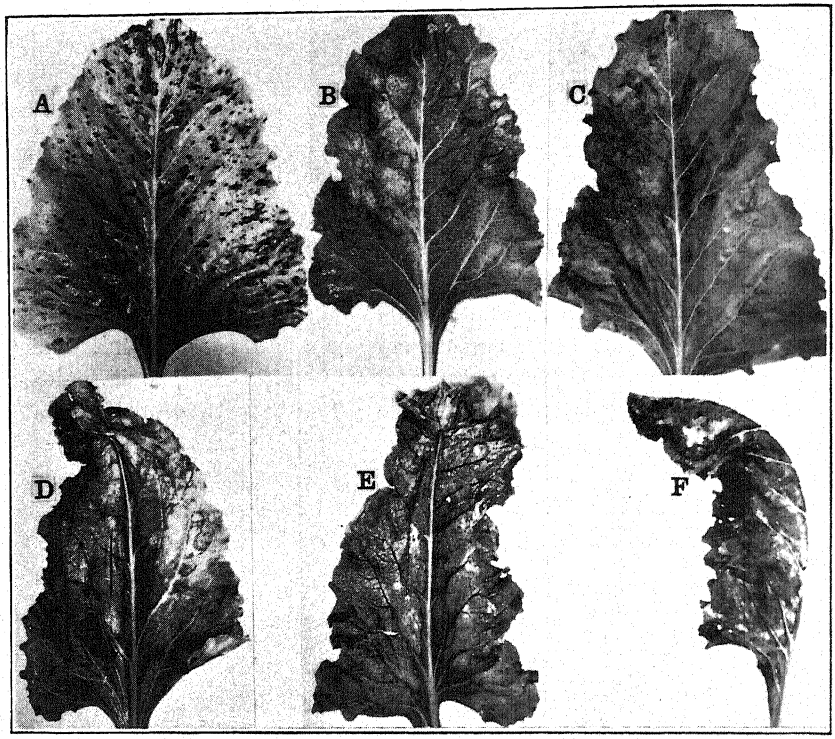


FIG. 1. Types of leaf injury characteristic of late blight. A. Spot type of lesions common on older and weakened leaves or of the milder form of disease on the more normal leaf. B, C, D, E, F. Stages in collapse of leaf blade, indicating the more severe expression of late blight both on the older and younger leaves. F. Upward and inward curving of the collapsed leaf blade. Note green and apparently normal tissue adjacent to the midrib and larger veins.

blade (Fig. 1, D, E). It is not uncommon to find both the small and large lesions on the same leaf.

The smaller lesions appear associated with the milder form of late blight, although, with the continued progress of the disease, these smaller necrotic areas may coalesce until the final result is much the same in appearance and in effect as when the larger leaf areas are suddenly affected. In the most severe types of tissue destruction a narrow margin of green and apparently healthy tissue remains intact adjacent to the main lateral veins and midrib, frequently lining the entire veinal system (Fig. 1, D, F; Fig. 2, A). In nearly all cases the surviving skeletal structure, which continues to support the dry necrotic tissue *in situ*, together with the leaf petiole, remains rigid for a considerable time, thus providing a characteristic feature of late blight (Fig. 2, A B). Under conditions of complete collapse

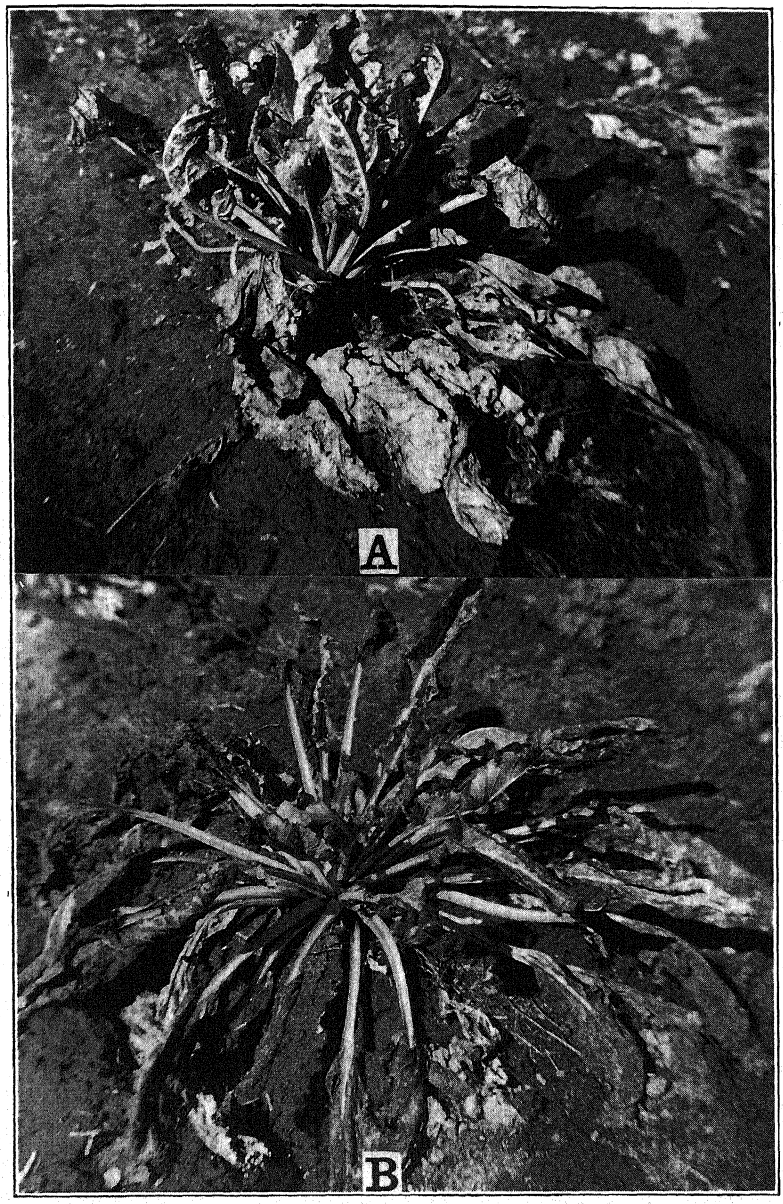


FIG. 2. Individual beets showing late blight. A. Advanced stage of the disease. Older leaves are prostrate and all inner leaves are more or less affected. Note characteristic collapse of tissue between larger lateral veins and upward curving of leaf blade, also rigid, upright position of petioles. B. The advance of the disease on older leaves while heart leaves remain apparently healthy.

of the leaf blade, the petiole may remain upright and rigid as long as the major portion of it continues green (Fig. 2, A, B). With weakening of the petiole collapse of the entire leaf occurs. Severely affected leaves may turn either upward and inward over the midrib (Fig. 2, F) or may become definitely recurved.

In the very earliest or initial stages of tissue collapse in either the small or the large lesional areas the affected tissue exhibits a Biscay Green[4] (Pl. XVII, 28′ i) or Rainette Green (Pl. XXI, 27″ i) water-soaked appearance. The green shades rapidly disappear, giving place to the darker shades of gray and brown. In wet weather the grays predominate, with lesser tendency toward drying of leaf tissue, the color passing through shades of Deep Olive Gray (Pl. LI, 23 h) to Dark Olive Gray (Pl. LI, 23‴ i) or Iron Gray (Pl. LI, 23⁗ k). Heavy dew apparently intensifies the darker shades. The development of the disease during dry periods results in a predominance of the tan or brown colors, which may vary from Cartridge Buff (Pl. XXX, 19′ f) to Olive (Pl. XXX, 12″ n) or Dark Greenish-Olive (Pl. XXX, 23″ m). Frequently with long exposure to direct sunlight the necrotic tissue areas are bleached to a Cartridge Buff (Pl. XXX, 19″ f) or Cream Buff (Pl. XXX, 19″ d).

When exposed to sunshine the collapsed tissue rapidly dries out, becoming membranous in texture and very fragile. The dead tissue is ruptured with but slight pressure, although it seldom, if ever, drops out. Winds readily whip the dried necrotic tissue to pieces, frequently giving the leaf an appearance as if riddled by hail.

In the development of the disease the outer leaves are affected first (Fig. 2, A, B; Fig. 3, A, B). As the disease progresses inward, successive whorls succumb until, in the late stages, all the leaves of the beet may collapse and finally appear as a brown or black mass attached to the crown (Fig. 2, A, B; Fig. 3, A, B). In mild cases of late blight, and especially where the beet becomes affected late in the season, this final stage does not always develop, and not infrequently at harvest time diseased beets are found with the outer leaf whorls collapsed and prostrate, while the center whorls appear to be quite healthy (Fig. 2, B).

In the field late blight may develop on single beets scattered among healthy neighbors or in somewhat localized areas which appear to radiate outward from a central spot; in general, this occurs most rapidly in the direction of the irrigation rows. Again the disease may appear more or less suddenly over a considerable portion of or even over the entire field, giving a definite blight aspect. The appearance of a field in its later stage of disease development is shown in figure 3, A, B.

[4] Color names beginning with capitals are those of Ridgway's Color Standards and Nomenclature. Washington, 1912.

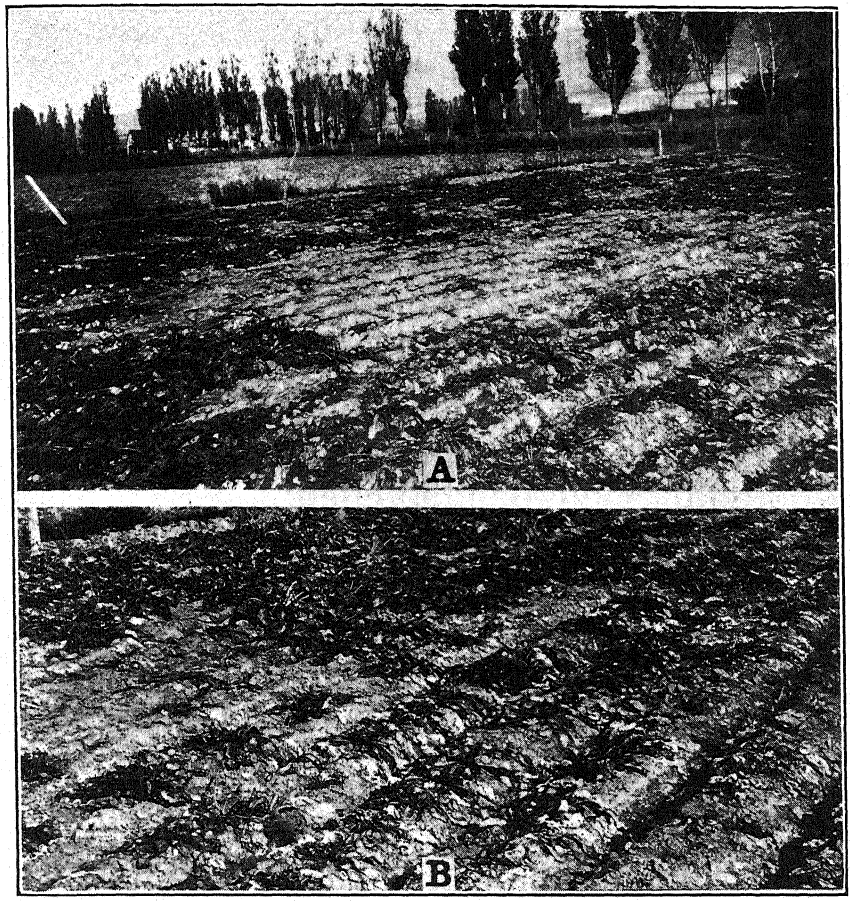


FIG. 3. Views of 2.5-acre field at Greenville, Utah, showing nature and degree of late-blight destruction during 1921. For details see text, also table 3. This field yielded 2.25 tons an acre in 1921 but was entirely free from disease. A. General view of field. B. Detailed view of an affected area.

If, during the progress of leaf destruction, the taproot is removed from the soil, lateral rootlets will be found seriously affected. Even in the early stages of leaf collapse many rootlets may be dead, dried up, and frequently blackened. As rootlet destruction progresses the taproot loses its turgidity and may remain in a wilted state for a considerable period. In dry seasons, as in 1921 (Table 4, B), this condition of the taproot may obtain until harvest with the majority of the affected beets. From such studies made during the various epidemic years it is clearly evident that under Utah conditions leaf and rootlet collapse may continue to completion prior to the initiation of taproot decay. On the other hand, with conditions of high or

excessive soil moisture due to late rains or late irrigations, rots are initiated usually by *Phoma betae* and a high percentage of the taproots may be partially or completely decayed before harvest.

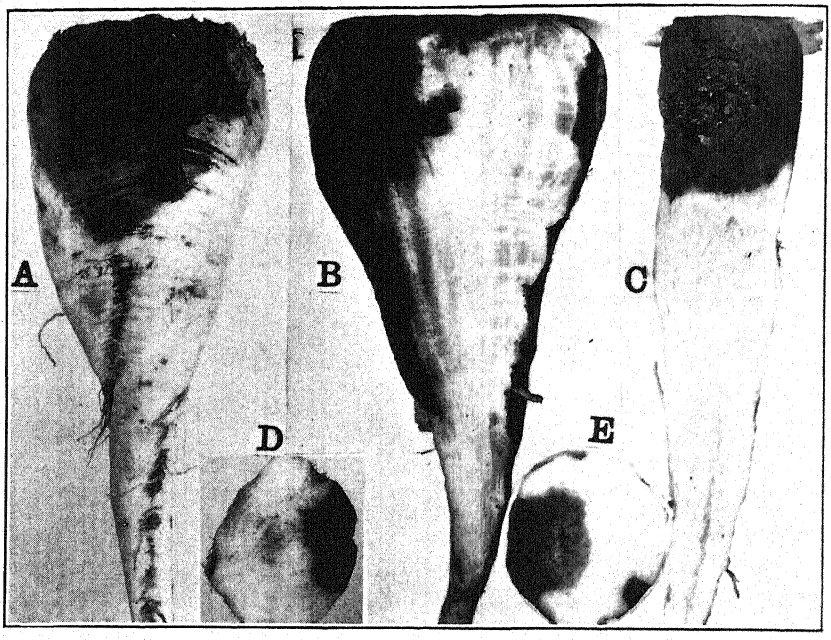


FIG. 4. Types of the dry rot of the sugar beet produced by *Phoma betae*. A. External appearance of rot area. B. Longitudinal section showing results of a number of lateral points of infection. C. Longitudinal section showing results of crown infection. Note cavities with included mycelium characteristic of advanced decay in dry soil. D and E. Cross sections of roots affected with dry rot.

In Utah the external rot lesions on the taproot do not assume definite shape, although the line of diseased tissue is sharply delimited from the adjoining apparently healthy tissue (Fig. 4, A, B). There may be one or several lesions of similar or varying size which have resulted from crown or petiolar infection or from independent lateral infection from one to several inches below the crown. A combination of the two types of lesions may develop on the same root (Fig. 4, B). The color of the external diseased areas varies from Sepia (Pl. XXIX, 17″ m) to Blackish-Brown (Pl. XLV, 9 m 3). The decayed tissue is ordinarily firm, and, except when dried out, the beet retains its normal shape.

Frequently, with increased fungous activity and subsequent drying out of the affected tissue, characteristic cavities are formed which are completely filled with mycelium (Fig. 4, C). The affected tissues, including

the rings or vascular bundles, become uniformly discolored. The early stages of internal breakdown, if caused by *Phoma betae,* are generally of a Deep Brownish Drab (Pl. XLV, 9‴ i), although as the rotting progresses the color changes to Light Seal Brown (Pl. XXXIX, 9‴ m) and finally to Blackish-Brown (Pl. XLV, 9⁗ m). Tissue decay is essentially of the dry-rot type.

IDENTITY AND DISTRIBUTION OF LATE BLIGHT

According to Krüger (15), Kühn and Schacht noted and investigated the so-called *Herzkrankheit* in 1850 and 1860, respectively. However, Gäumann (9) is of the opinion that the first information concerning a sugar-beet disease, the description of which might be considered as identical with that for *Herzkrankheit* or *Herz-und-Trockenfäule* as now known, originated from Frank in 1897 and Stift in 1900. At any rate, these apparently synonymous terms are descriptive of a disease or a disease complex that ranks with the oldest of sugar-beet diseases and parallels very closely the symptomatology of a late blight as it occurs in Utah and also the trouble referred to as dry rot or Phoma root rot by Edson (2) and other American writers. The conclusion that the disease complex, so designated, is considered as an expression of the same set of factors on the two continents is further emphasized by the recent recognition of its two-phase nature by workers in Europe and by the present writers in Utah. The fact that on the two continents the trouble is intimately related in its epidemic form to dry seasons further confirms the possibility of its identical etiology.

With a clear recognition of the blight stage of the disease as a condition independent of root rot, except as a predisposing factor, it becomes evident that the various terms such as dry rot, Phoma rot, *Trockenfäule,* etc., fail to characterize the disease complex as now understood. In fact, these names are misleading unless restricted in meaning to the root-rot stage of the trouble. The writers here propose late blight, as formerly used by the senior writer (20), to designate the blight stage of the disease in Utah, realizing that rotting of the taproot, which the older terminology characterizes, may or may not follow as a part of the pathological picture.

Late blight, if identical with *Trockenfäule* and possibly *Herzkrankheit,* is world-wide in its distribution. It has been reported to the Office of Mycology and Disease Survey, United States Department of Agriculture, from the British Isles, from all the countries of continental Europe where sugar beets are grown, and from Japan and Korea. It also occurs in every State in the United States where beets are grown commercially.

OCCURRENCE OF LATE BLIGHT IN UTAH

Late blight appeared in epidemic proportions in Utah in 1919 and 1921 and in isolated fields in various localities in 1917, 1924, and 1926.

In 1919 the disease was destructive in the beet-growing districts of Cache, Boxelder, Utah, Sevier, and Salt Lake counties. In Cache Valley 30 to 40 per cent of the beet fields were reported affected, the degree of infection varying from a fraction of 1 per cent in some fields to total destruction in others. Root rot during September and October became a conspicuous feature of the disease and figured heavily in decreased yields. A large number of fields were unfit for harvest. Root rot also was serious in the storage piles and became especially destructive in beets stored for seed purposes. Seed production was suspended in Utah owing largely to the storage troubles encountered in 1917 to 1921.

In 1921 late blight was more serious than in 1919 and was reported from nearly every sugar-beet-growing district in Utah. As in 1919, Cache Valley again suffered more severely than other parts of the State, although the trouble was severe in Boxelder and Sevier counties and also in the Spanish Fork and Payson districts of Utah County. Approximately 100 fields in Cache Valley were blighted, and in many fields the crop was a complete failure. Table 1, which represents data from a 2.5-acre field in Greenville (Cache County), Utah, gives some idea of the degree of destruction. This field was not irrigated excessively in September and October and the loss from root rot was a minor cause of decrease in yield. Its average acre yield was 2.25 tons.

TABLE 1.—*Condition of beets in three representative rows of a 2.5-acre field in Greenville, Utah, showing late blight in the epidemic year 1921*
(See also Fig. 3, A and B)

Row No.	Total No. beets	No. of beets diseased		Beets showing decayed lesions No.	Diseased beets showing decayed lesions (%)	Healthy beets No.	Healthy beets (%)
		Tops not dead	Tops dead				
1	219	49	106	9	5.4	64	29.2
2	280	62	156	16	6.8	62	22.1
3	269	58	152	19	8.3	59	21.9

Cache Valley, as a whole, suffered a reduction of 3.35 tons below the average yield or 4.94 tons below the average for the high-yield years. Much of this loss was directly attributable to late blight. The blight stage of the disease was more severe than in 1919, although the rotting of the taproot was much less conspicuous and important. By far the greater part of yield reduction was occasioned by the production of small beets and by the loss

of water from the beets after the leaves had collapsed rather than because of root decay. Rotting of the taproot was a serious source of loss only in fields where later in the season a high soil-moisture content was maintained by irrigation.

Both in 1920 and 1922, following the epidemic years of 1919 and 1921, the disease completely cleared up, leaving no signs of the previous years' epidemics.

In 1917, while possibly not of epidemic proportions, late blight did manifest itself in rather severe form in isolated areas in Cache and Boxelder counties. Reports of its occurrence also were received from Sevier County and from the Payson and Spanish Fork districts of Utah County. Losses were heavy in many fields in these counties.

During 1924 and 1926 late blight occurred only in fields where poor cultural operations had exposed the beets to vigorous drought conditions either through poor tilth, lack of fertility, or lack of proper irrigation. Neither 1924 nor 1926 could be considered as blight years and losses from late blight were small compared with decreased yields caused by the curly-top disease induced by the feeding of the leaf hopper *Eutettix tenellus*. Both 1924 and 1926 were outstanding for the presence of this latter trouble. In fact, 1926 stands out as the year of the most severe damage in the history of the sugar-beet industry in Utah. Neglect of beet fields owing to the progress of curly top was general and was probably the most important factor in inducing late blight both in 1924 and 1926. Knowlton (13) in discussing curly top in Utah states: "Curly top was more severe in 1926 than in 1924 and many fields in the northern part of the State were very much neglected." He estimated crop yields at 7 to 10 tons and attributed the decrease in yield primarily to curly top.

In 1929 late blight became important in a few fields in Cache Valley. Twelve of these fields were studied in considerable detail and will be discussed in a later portion of this paper.

FACTORS INFLUENCING THE OCCURRENCE OF LATE BLIGHT

Frank (4) in 1892 reported that weather had no influence on the spread of the dry or heart rot in Germany. By 1893 he (5) had altered his views and announced that dry soil and dry weather did favor the appearance of the disease, although he considered these factors as secondary in importance to the organism *Phoma betae*. In 1895 Frank (6) again emphasized the relation of dryness to the disease in July and August but held that dryness alone would not cause the trouble. He noted that excessive soil moisture appeared to inhibit the actions of *P. betae*.

Hollrung (10), in 1893, called attention to the fact that heart rot is most prevalent in dry seasons and in clay soils underlaid by an impermeable

stratum. Lack of water and unfavorable soil conditions rendered the crop more subject to attack by *Phoma betae.*

In 1909 Schander (22) reported that the disease always appeared after a period of great heat and dryness.

Sorauer (24) noted that the dry rot was favored in its development by high soil temperatures and lack of sufficient water for normal plant growth. The disease, he stated, appeared generally in mid-July if hot, dry weather persisted.

Labbé (17) also considers the disease to be more severe during dry periods, and Schander (22, 23) recognized water supply as of primary importance in connection with the development of the disease in Germany and points out the seriousness of dry rot in the Province of Posen as compared with central Germany is due to the fact that the former has a more unfavorable climate with more frequent and destructive dry periods in early summer, especially in May and July.

In 1913 Kappeli and Morgenthaler (11) stated that with restricted respiration and assimilation of the host plants, due to drought, penetration by *Phoma betae* was favored.

Edson (2), in a discussion of the perpetuation of *Phoma betae,* observes that the root may be susceptible to infection during periods of low vitality induced by unfavorable environment. However, he concluded that under conditions obtaining in Wisconsin and Colorado in the summer and fall of 1913 Phoma rot developed under conditions of excessive moisture and of severe drought in the respective States.

According to Nakata, Nakajima, and Takimoto (19), "snake-eye" in Korea, a disease associated with *Phoma betae,* is one of the most serious beet diseases and breaks out in sandy soil or during dry seasons.

From a study of the disease in Utah, Richards (20) concludes that, in its epidemic occurrence, it is closely correlated with abnormally low precipitation during the months of June and July and may be induced in isolated fields by local drought conditions of the soil.

Brandes (1) noted that Phoma root rot in the United States is influenced largely in its development by weather conditions, excessive moisture and high temperature being the principal factors favoring it.

Gäumann (9) in his investigation of water in its relationship to the disease found that many soils, in which diseased beets had developed, possessed a smaller water-holding capacity than the soils in which healthy beets had grown to maturity uninfected. In other fields the question of water-holding capacity seemed to be less important as a primary contributing cause of the trouble. He observed that wet, cool weather inhibited, while hot dry weather favored the disease. Gäumann holds that while the

water relationship is important it is of secondary importance when compared with soil reaction and the calcium and magnesium carbonate content of the soil. Occasionally, however, it may be of primary importance.

Studies by Krüger and Wimmer (16) indicate that while the heart and dry-rot disease may appear at different periods of growth of the sugar beet, it develops most frequently after the time of the most luxuriant leaf growth. This, they stated, occurs at the end of July or early in August in dry, rainless summers.

Murphy (18), in 1927, believed the disease was associated with certain light soils liable to suffer from drought and in certain cases with reclaimed soils of a peaty nature.

In 1928 Esmarch (3) calls attention to the physiological nature of the heart and dry rot of the sugar beet and points out that the disease occurs during July and August in dry summers and under drought conditions of the soil where the water balance of the plant is so disturbed as to become an important factor. According to this writer, soils that dry out readily, heavy soils that crust, and soils underlaid with rocky subsoil or that permit an easy run-off greatly favor the development of the trouble. The disease is intensified also by high alkaline content of the soil, especially by the abundance of lime, and is lessened in its severity by the application of amendments containing phosphoric acid and potassium.

Conditions favoring late blight in Utah agree closely with those depicted by recent European workers to be responsible for heart and dry rot in Europe. Climatic factors, particularly the distribution of rainfall, also local soil and cultural conditions, are involved.

Data relating to rainfall, yield, and the incidence of late blight in the Logan beet district over the period from 1915 to 1929 are included in table 2. A glance at columns 1, 17, and 18 shows clearly that certain seasons are definitely unfavorable for beet production. In 1917, 1919, 1921, 1924, and 1926, yields in the Logan area and in Cache Valley fell far below the average yields for these districts. Available data not included in the table show that a similar decrease in yield resulted during these years for Utah as a whole. During the same years in which beets yielded poorly (1915–1929, inclusive), poor potato yields were obtained (Table 2, column 20). A study of column 19 further discloses the fact that the two years of low yields in sugar beets and potatoes were those in which late blight occurred either in epidemic form or to a lesser degree in isolated areas throughout Utah. The factors thus responsible for low yields and late blight are evidently the same and are operative apparently over a wide area.

Recent studies have disclosed the fact that a close correlation exists between the distribution of rainfall and the incidence of occurrence of both

TABLE 2.—*Precipitation record as related to the occurrence of late blight in the Logan district and Cache Valley, Utah, during years 1915 to 1929, inclusive*

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Year	Monthly precipitation												Annual Ppt.	Total Ppt. for June, July, August	Total for September, October	Yield Logan district (tons per acre)	Yield for Cache Valley (tons per acre)	Occurrence of late blight	Potato yield for State of Utah (Bu. per acre)
	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.							
1915	1.06	1.32	0.59	1.49	3.28	1.12	0.22	T	3.44	0.05	.....	0.55	19.59	1.34	3.49	12.01	11.66	None	125
1916	2.61	2.62	2.17	1.73	0.91	0.88	0.08	0.20	0.10	3.78	0.80	2.89	18.77	1.16	3.88	12.30	10.96	None	180
1917	0.91	4.51	1.88	2.84	4.21	0.48	0.48	0.00	1.34	0.07	0.77	0.65	18.14	0.96	1.41	7.96	9.22	Light	189
1918	3.15	2.33	1.80	0.80	1.82	0.44	1.14	0.36	1.22	2.56	0.94	0.35	16.91	1.94	3.78	13.04	13.63	None	180
1919	0.02	1.88	0.74	1.62	1.20	0.00	0.31	0.40	2.88	4.43	0.73	1.49	15.70	0.71	7.31	9.37	11.09	Epidemic	136
1920	0.26	1.24	2.73	3.08	0.94	0.28	0.19	1.38	1.57	4.70	1.36	1.51	19.24	1.85	6.27	12.01	12.92	None	189
1921	1.48	1.22	2.77	3.64	1.51	0.01	T	0.74	0.34	1.28	.....	.....	18.33	0.75	1.52	8.04	8.04	Epidemic	161
1922	1.45	1.85	1.70	1.12	1.65	0.70	0.77	1.28	0.17	0.37	0.35	2.55	15.16	2.75	0.48	12.20	11.42	None	197
1923	2.71	0.48	0.96	3.10	1.53	1.81	0.58	0.64	1.30	2.14	0.87	0.79	16.91	3.03	3.40	13.69	12.66	None	168
1924	0.28	0.73	2.20	0.44	0.97	0.36	0.42	0.07	0.07	2.60	1.22	3.18	12.42	0.82	0.96	7.45	7.03	Light with curly top	136
1925	0.56	1.61	2.26	1.22	1.45	2.06	0.32	0.86	2.15	0.43	1.86	1.18	16.32	3.24	2.58	15.95	16.29	None	180
1926	0.88	2.69	0.69	1.32	2.18	0.24	1.55	1.13	1.85	0.43	1.97	1.03	15.97	2.92	2.28	9.16	9.29	Light with curly top	145
1927	1.15	1.96	1.85	2.82	2.73	0.38	0.67	0.80	1.87	1.37	1.68	0.93	18.38	1.85	3.24	14.24	13.57	None	135
1928	0.70	0.45	1.99	0.92	1.39	1.48	0.37	0.02	1.09	.....	.....	0.75	.....	1.87	1.09	13.18	12.62	None	144
1929	1.87	1.12	2.12	2.42	0.32	1.46	0.22	0.45	2.91	1.82	0.93	.....	15.56	2.13	4.73	11.92	12.18	Light in Cache Valley	185

the low yields and the late blight. With the exception of 1926, the years of low beet yields were those with abnormally low precipitation for the months of June, July, and August, in each case falling below the total of 1 inch (Table 2, columns 15 and 19). Except for 1926, the disease appeared only in years of low rainfall during these months. In 1919 and 1921, epidemic years, precipitation during June, July, and August was especially low. In 1921, the most severe of all years for late blight, 0.01 inch fell in June, but a trace in July, and 0.74 inch in August, or a total of 0.75 inch for the three critical months in the growth cycle of the beet. During 1919 no rain fell in June, 0.31 inch in July, and 0.4 inch in August, totaling 0.71 inch for the three months. Both in 1919 and in 1921 severe drought occurred during the critical period in which the young beet was forced to establish itself after thinning. The effect of low precipitation is expressed both in the amount of disease and in the decreased yield. Late blight in 1917 and 1924, though less abundant, bears an equally prominent relationship to the low precipitation during the three months of June, July, and August.

In 1919 rot of the taproot appeared to the casual observer as the most prominent feature of late blight, being frequently initiated during the very earliest stages of leaf collapse. In a number of fields in Cache Valley decay of nearly 100 per cent of the roots resulted. In a high percentage of these fields, owing to the extent of root rot, the crop was not harvested. The drought period during June, July, and August, responsible for the severe blight of beets, was followed during September and October by heavy rainfall. In these two months 7.13 inches fell, which was 4.4 inches above, or approximately two and a half times normal September and October precipitation for the Logan district. This excess rainfall together with irrigation kept the soil continually wet and definitely favored the decay of the beets.

Even though late blight was more severe in 1921 than in 1919, root rot was noticeably less important. The degree of root decay in proportion to affected beets is indicated in table 1, column 6. Under field conditions, wherein excess irrigation was not a factor, rotting of the taproot was definitely inhibited and large numbers of beets without decay were harvested, the leaves of which had completely collapsed two to three weeks prior to digging. The precipitation record for 1921, when compared with that of 1919, is instructive. The severe drought during June, July, and August, 1921, continued through September and October. The total precipitation for these two months amounted to 1.62 inches, approximately one half normal as compared to $2\frac{1}{2}$ times the normal for the corresponding 2 months in 1919. It is interesting in this connection to recall the observations of

Brandes (1) and Edson (2) that root rot is favored by excessive moisture and high temperature. Neither of these writers indicates that his observations were made over the entire season, nor is there any indication that blight was recognized by either of them as a distinctive feature of the disease separate from root rot. Edson states that root rot developed under conditions of severe drought in Colorado the same season that it developed in Wisconsin under conditions of excess moisture. It is during the two months of September and October that the taproots, reduced in vitality by late blight during June, July, and August, are most exposed to the factors of decay. The degree and rate of decay appear to be determined by the amount of moisture in the soil. Whatever the explanation of the difference in the quantity of decay in the two years, it is clear from the studies in 1924 that root rot is not a necessary part of the late-blight picture but is initiated as a result of the conditions responsible for late blight.

Studies and observations in 1917, 1919, 1921, and subsequent years have disclosed a number of soil and cultural conditions which definitely favor the development of late blight. Delayed irrigation has frequently been noted as an important factor in prolonging the early drought period and thereby tending to induce the disease. In one instance in 1921, in a field otherwise uniform in cultural relations, five rows were left unirrigated for two weeks after the first irrigation of the other portions of the field. Beets in these five rows became so badly blighted that they were left unharvested, while only local areas in the early-watered portion were seriously damaged. Similar instances have been noted in a considerable number of fields where early irrigation had been delayed or in spots in fields inaccessible to adequate irrigation water.

Surveys in 1921 and 1929 have also shown a close correlation between the incidence of late blight and previous cropping, especially as the latter relates to soil tilth, soil fertility, and the humus content of the soil. In 1921 the most severe type of late blight occurred on soil following cereal crops or for the first time in sugar beets for a number of years. Of the 100 fields studied in Cache Valley in 1921, 23 fields which exhibited the trouble in its most severe form had been planted to sugar beets immediately following either alfalfa or cereal crops. The condition of tilth in these fields was generally poor, and, during the epidemic, few fields of this character escaped the disease. The greater percentage scarcely paid for harvesting; many were left unharvested.

In September, 1929, eleven fields were located in Cache Valley, Utah, in which late blight was prevalent. As the valley was otherwise free from the trouble, the situation was unique for investigation. An intensive study was made involving the problems of tilth, crop sequences, organic content of the

TABLE 3.—*Showing soil and cultural conditions which influenced the occurrence and development of the late blight of the sugar beet in Cache Valley during the non-epidemic year 1929*

Field	Acreage	Soil type	Cropping history			Fertility	Irrigation	Diseased (%)	Additional observations
			1926	1927	1928				
1	1.5	Black sandy loam	Grain	Grain	Potatoes		Became too dry	25	Allowed beets to become too dry
2	10.5							Localized	Disease limited to area where well-traveled road passed through the field
3	4	Black sandy loam	Grain following pasture	Beets	Beets		Two irrigations		Field permitted to become very dry
4	3.5	Black sandy loam	Beets	Grain	Grain	Not manured		20	Crop allowed to get too dry
5	3.5	Clay loam	Alfalfa	Alfalfa	Alfalfa	Manured winter 1928	Three irrigations	10	Disease appeared about September 1, adjoining 5-acre field 3 years in beets, free
6	4	Gravelly loam	Alfalfa	Alfalfa	Alfalfa	No manure	Four irrigations	10	Field permitted to get too dry before first irrigation
7	4.5	Heavy clay	Old alfalfa	Alfalfa	Alfalfa	Crowned 1928, manure	Four irrigations	10	Long irrigation rows (0.25 mile long). Disease only on lower part of field owing to inadequate irrigation
8	3		Alfalfa old	Alfalfa	Alfalfa		Sub-irrigated	25	Beets became dry on new land; 12-acre field adjoining, which had been in beets for 4 years free from disease
9	3	Sandy loam	Alfalfa since 1922	Grain	Grain	Manured 1928	Three irrigations	20	Disease developed in the areas given extra heavy application of manure
10	6	Clay pasture	Pasture	Pasture	Pasture		Sub-irrigated	20	Part of same field in beans 1928 free from disease; remainder diseased
11	4.5	Sandy loam	Alfalfa 1920	Alfalfa	Potatoes	Manured 7.5 tons 1928		90	Disease most severe on lower end of field; rows too long for effective irrigation of lower end of field

soil, and dates, frequency, and type of irrigation. The results of the studies are summarized in table 3. It is evident that late blight in Cache Valley was limited during 1929 primarily to soils planted to beets following alfalfa, cereals, and pasture, or to potato land following grain or alfalfa on which irrigation had been neglected. The disease apparently was not associated with any particular soil type. Soil topography was noted as an important factor only in determining the quantity of irrigation water available for local areas within the field.

In addition to the effect of previous cropping the lack of water in sufficient amounts applied at such intervals as to maintain uninterrupted plant growth appeared as a most important factor inducing late blight. It is probable in this connection that excessive quantities of crude or undecayed organic matter in the form of alfalfa roots and crowns (Table 6) and constant cropping with cereals so adversely affect soil tilth as to make for poor water-retaining power, thereby contributing to the disease. Where alfalfa was first crowned in the fall and then plowed under in the late fall or early spring, the succeeding crop was always severely affected. Heavy manuring of alfalfa land prior to or following plowing also intensified the late-blight situation. In the study of these few fields it was found that a delay of the first irrigation and excessively long irrigation rows were definite factors contributing to late blight.

Accumulated evidence indicates that heavy first irrigations following the long period of drought in June and early July are important in blight production. The disease seldom, if ever, appears in its typical form prior to the application of water but frequently develops rather rapidly thereafter, especially if the soil is heavily irrigated. In this relation particularly in respect to the suddenness with which lesions develop in the leaf tissue, late blight closely resembles white spot of alfalfa as described in 1928 by Richards (21). Radical fluctuations in irrigation practice also seem to be an intensifying factor.

Soil reaction has been observed to play an important part also in inducing late blight in Utah. In general, in epidemic years the disease is more severe on alkaline soils than on soils known to be less alkaline. Soils rich in lime favor the disease.

The relative importance of soil reaction to the occurrence of late blight has long been recognized. Frank found the disease more prevalent in 1896 in Silesian Germany on soils that had been too heavily treated with Chile saltpeter. Kiehl (12) concluded from his studies that the disease was due to excessive concentrations of soil nutrients rather than to *Phoma betae*, as others had claimed. Wilfarth and Wimmer (29), by use of pot experiments, determined that high soil alkalinity favored heart rot and dry rot.

Krüger (14) observed that poorly drained and nonaerated soils with a strongly alkaline reaction favored the development of the disease. Fertilization with sodium nitrate increased, while gypsum and ammonium sulphate checked it. Störmer (28), recommended as a control measure the neutralizing of the alkaline condition of the soil in order to render it physiologically acid. Schander (23), however, was unable to confirm Störmer's results and claimed that ammonium sulphate in large quantities favors root rot.

Gäumann (9) calls attention to the fact that the disease has not appeared in Switzerland on acid soils and that it is seldom found on neutral and slightly alkaline soils. However, with a soil pH of 7.6 or greater, the disease becomes severe. The intensity and frequency of the disease increase with an increase in pH. Complete loss of the crop may be expected in soils with a pH of 7.7 or greater. The critical pH point varies with different fields. A soil pH of 6.6 was found to be inhibitory, in so far as disease development was concerned.

Krüger and Wimmer (16) observe that the disease may appear at the end of July or early August in dry summers, but later, in rainy summers, in connection with the early or late increase in alkalinity of the soil solution. The nature of this pH increase may determine in large measure whether the disease will appear in light or severe form.

Murphy (18) argues that since this type of crown rot occurs mainly on alkaline soils and as alkalinity is principally determined by the amount of lime present, it may be said that for practical purposes it is confined to soils containing a considerable amount of lime.

Phosphoric fertilizers, according to Esmarch (3), seemed to inhibit the disease by reducing the alkalinity, while Chile saltpeter and potash (in the form of kainit) and calcium carbonate increased the disease. An alkaline soil reaction, he states, was known definitely to favor disease development.

On October 10, 1929, six soil samples were collected from each of five Cache Valley fields in which late blight was prevalent, and pH determined. The average pH values for each are shown in table 4. Values obtained agree well with those found by Gäumann (9) to favor late-blight development. These values are further fairly characteristic of Cache Valley soils as a whole, which, together with their high lime content, offer a possible explanation of the seriousness of late blight in the valley. Boxelder County offers a soil condition similar to that of Cache Valley and the Payson and Spanish Fork districts in Utah County, on which late blight was serious both in 1919 and in 1921, provide a soil rich in calcium. Sevier County, second only to Cache Valley in the severity of late blight, is generally known for its alkaline soils. The exact part played by soil reaction in the develop-

TABLE 4.—*Soil pH determinations of five late-blight fields in Cache Valley, Utah, 1929. (Samples collected October 10)*

Field No.	Location	Average pH values (6 soil samples taken from each field)	Probable error
1	South Logan, Utah	7.511	0.039
2	Hyde Park, "	7.740	0.54
3	South Logan, "	7.843	0.056
5	South Logan, "	7.455	0.079
7	Weston, Idaho	7.585	0.112

ment of late blight remains a problem for the future. The disease may and does occur on soil not excessively alkaline and not rich in lime, although under certain conditions both may be considered as intensifying factors.

Studies and observations in Utah since 1917 and especially those made in 1919 and 1921 indicate that late blight is more severe in late-planted than in early-planted fields. Poor cultivation and excess weed development were also correlated with late-blight occurrence.

NATURE AND CAUSE OF LATE BLIGHT

The exact nature and cause of late blight, whether of a parasitic or non-parasitic origin, have remained a major question in the minds of workers ever since the trouble has been recognized as a specific disease. The parasitic theory first came into prominence through the early work of Frank and has had its supporters both in continental Europe and in America. In general, advocates of the parasitic theory have attributed the trouble to *Phoma betae,* owing largely to the more or less constant association of this organism with the final root decay accompanying the late blight. Edson (2) goes so far as to suggest that this fungus, after attacking the beet in the seedling stage, remains in a dormant or semidormant form in the tissues of the growing beet and, finally, with decreased vitality of the host tissue, resumes activity, bringing about the dry rot so characteristic of the late-blight trouble. So far as the present writers are able to learn, there exists no experimental proof of such a relation.

Isolation work in Utah in 1919 and 1921 showed *Phoma betae* to be constantly associated with the rotting of the taproot following late blight. The same fact was experienced in 1929, and with but few exceptions *P. betae* has been found associated with the root-rot stage of the trouble in this and

TABLE 5.—*Results of isolations from sugar beet root showing the typical root rot that follows or accompanies the late blight. Diseased beets were collected during 1921 from various parts of Utah and Idaho*

Source of beets	No. beets	No. isolations	Isolations showing		No. miscellaneous organisms	No. sterile
			Phoma betae	Fusaria		
			1921			
Greenville	11	48	38	2	7	1
West Field (Logan)	6	29	29			
Garland	12	48	41	2	2	3
North Logan	16	64	49		8	7
South Logan	12	48	42	1	3	2
Honeyville	8	16	12	2	2	
Preston, Idaho	4	16	14	2		
			1929			
South Logan	24	72	57			15
" "	24	72	60			12
Hyde Park	24	72	68			4
Weston, Idaho	24	72	45			27

other countries. On the other hand, no one has yet claimed to be able to produce experimentally late blight or root rot by inoculating strains of *P. betae* into the tissues of healthy, growing sugar beets. In both 1921 and 1929 the present writers obtained only negative results from a large number of trials in which *P. betae* was placed adjacent to both normal and wounded beet roots as they grew in the soil. Where beets were harvested and placed either in moist chambers or under favorable conditions in storage, decay from pure Phoma cultures was readily obtained.

Under European conditions, particularly in Germany, *Phoma betae* becomes associated with the leaf and petiole destruction characteristic of the early stage of the disease. In America, and especially under conditions as found in Utah, no such association occurs. Instead, the affected leaf tissues, if taken in the very early state of tissue collapse, are found entirely sterile. Isolations were made in 1921 from 224 late-blight leaves in the early stage of tissue collapse representing in all 112 sugar beets. Out of the 224 leaves, 219, or 95.5 per cent, were sterile; five gave species of Alternaria. Table 6 gives additional and similar results obtained in 1929.

Nearly all workers have become convinced that, in general, *Phoma betae* is, at most, a weak parasite, capable of causing root rot or leaf destruction only after the normal resistance of the beet has been seriously impaired. Even Frank (7, 8), the most vigorous exponent of the parasitic theory, states that the parasitism of *P. betae* is associated intimately with, if not dependent upon, the predisposing effect of dry soil and dry weather.

TABLE 6.—*Results of isolations made from typical late blight leaves of sugar beet during the early stage of tissue collapse 1929*

Source of leaves	No. leaves	No. isolations	Organisms	Sterile
South Logan	24	72		72
" "	24	72		72
" "	24	72		72
Greenville	24	72		72
Hyde Park	24	72		72
Weston, Idaho	24	72	1 Alternaria	71

The absence of positive experimental evidence supporting the parasitism of *Phoma betae,* the frequent occurrence of late-blight condition unassociated with any root rot as reported from Utah and also by Hollrung (10) and others, together with sterile conditions of blighted leaves of late-blight plants, argue strongly against the parasitic theory of origin. Again, *P. betae* may become associated with other diseases which weaken the resistance of the taproot quite as readily as does the late blight. Other fungi, especially species of Fusarium, may be the first invaders under late-blight condition, even to the exclusion of *P. betae.*

The more recent observations and research support definitely the nonparasitic concept of the origin of late blight. A number of workers have consistently maintained that late blight is definitely a nonparasitic disease, induced through some maladjustment of the plant to its physical environment. Various theories explaining these maladjustments have been proposed, although the views concerning the more exact nature of the etiological factors have been and still are extremely diverse.

As previously indicated, a number of workers, Gäumann (9), Krüger and Wimmer (16), Murphy (18), and Esmarch (3) attributed the trouble to an unfavorable soil reaction, especially one tending toward alkalinity. Murphy (18) states that for practical purposes it may be said that late blight is confined to soils with considerable lime; Esmarch (3), while emphasizing drought as the primary factor, persists in his emphasis of alkalinity as a contributing cause. In his suggestions on control this writer states that lime and liquid manure are to be avoided and that preference should be given to acid fertilizer.

Experiences in Utah indicate that soils of an alkaline reaction and possibly those heavy in lime may encourage the disease, although it is evident that these factors alone cannot be held as primary in their causal relation.

The organic content of the soil is of undoubted importance in inducing late blight, although just how it influences the development of the disease is by no means clear.

Excess organic matter, as shown by the observations made in Utah, favors the trouble. Gäumann (9) also points out the danger of high organic content, and Stift (26) reports observations of Kiehl and Schonsky to the effect that green manure and clover cropping are both factors to be avoided where late blight is a menacing factor in sugar-beet production. On the other hand, observations of Esmarch (3), Stift (27), and Richards (20) have emphasized the importance of a lack of organic matter as a factor favoring late blight. In general, the disease in Utah during the epidemic years was most severe on compact soils low in organic matter, especially following a long period of cereal cropping. From such observations it would appear that the organic content of the soil is not a factor contributing to the disease directly, but possibly only indirectly, both when in excess or when absent, through its influence upon the water-holding capacity of the soil.

As to the direct bearing of the fertility of the soil on the production of late blight there appears a great diversity of opinion. Frank (8) found that fertilization with Chile saltpeter promoted the disease. Stift (26) reports late blight following the use of liquid manure and explains that the rapid early growth induced by liquid manure and saltpeter lessens the power of the plant to withstand subsequent drought periods and that such rapidly growing plants succumb to drought more readily than plants more retarded in their growth. Krüger and Wimmer (16) claim to be able to produce heart rot in culture vessels and maintain that the disease can be definitely controlled by the use of such fertilizers as will most nearly correct the alkalinity of the soil. As a result of their work these writers suggest that heart rot is due essentially to the absence or presence of certain soil constituents and, with suitable amendments in sufficient amounts, they can definitely overcome the disease. While the results of these latter workers are suggestive, there is no assurance that the disease produced in their culture vessels or the heart rot described by Gäumann is identical with the late blight as it occurs under such a variety of conditions in nature.

Stewart and Pittman (25) determined the existence of a definite relation between what they designate as "late root rot" and high soil fertility maintained by use of stable manure. These writers introduce for the first time the term late root rot and in no way relate it to any known disease of the sugar beet. Their description, which, while no doubt including late blight, is so general as to render identification impossible. In fact, it would appear that what they discuss is a root-rot complex possibly consisting of

the several known rots, even involving the damping-off or root blight of seedlings, which they claim by use of general field-survey methods to be able to recognize as late as the first of July. Owing to this evident confusion, the data provided by these writers add little, if anything, to the understanding of the late-blight situation.

The indirect or secondary relation of soil fertility to the late-blight situation during 1921 is clearly shown in the performance of the 2.5-acre field in Greenville during the subsequent season of 1922. As related (p. 296 of text; Fig. 3 A, B), the beets in this field were severely affected in 1921. In the autumn of 1921, after the beets were harvested, one third of the field was heavily fertilized with stable manure (20 loads per acre), one third lightly manured (6 loads per acre), and one third left unmanured. Beets were planted under comparable conditions in 1922 on all three divisions, and the cultural practices were made as nearly uniform as possible. No sign of late blight appeared on any of the three divisions during the entire season of 1922. The fertility of the one third left unmanured was no doubt lower in 1922 than in 1921, yet no disease was evident. The performance of this field was typical of the complete clearing up of the disease in 1922 on soils of all types either of low or high fertility.

High soil fertility may influence the development of late blight variously; either by directly and adequately supplying required nutrients, thereby resulting in greater vigor and possibly greater resistance of the beet to drought and other weakening influences, or equally indirectly, if obtained by the supplying of manures by increasing the water-holding capacity of the soil. As an immediate cause of late blight or as a universal cure (25), its influence is clearly challenged.

From a study of the literature it is evident that the late-blight problem is one of extreme complexity and that the peculiar condition or set of conditions directly responsible for the disease at the present time remains obscure. On the other hand, the majority of writers relate the disease either directly or indirectly to an unbalanced water relation in the plant, induced locally by dry soil or over wide areas by warm, dry weather. Even such contributors as Frank (5, 6, 7, 8), Gäumann (9), Krüger and Wimmer (16), Edson (2), and Murphy (18), who hold other factors as primary, recognize the water relation as an important preconditioning factor. Others, Hollrung (10), Kiehl (12), Richards (20), Schander (22, 23), Stift (26, 27) and Esmarch (3), hold the view of a more direct etiological relation of the shortage of soil and atmospheric moisture to the disturbed physiology of the sugar beet here described as late blight.

Stift (27), in writing the history of the heart and dry rot, summarizes as follows: "Practical experiences have shown that it is indisputable that the heart and dry rot is always a consequence of summer drought, and, as

a matter of fact, the amount of rain (in its sum and even more in its distribution in the months of July and August) above all else determines the occurrence of the disease." Kiehl (12) states from his observations that even though the disease develops during a wet period, "its beginning rests on a preceding period of drought." Esmarch (3) in 1928, as will be recalled, emphasizes not only the dry season but details the local soil and cultural relations which determine drought relations of the soil and subsequently the water balance in the plant. Schander (22) points out that the eastern provinces of Germany, especially Posen, are more subject to dry rot of the beet, owing, as he states, to "the more frequent and destructive dry periods in the summer characteristic of their continental climate."

SUGGESTIONS FOR CONTROL

During the time that late blight has been under observation, recommendations for its control have been quite as numerous and quite as divergent as the theories presented in explanation of its cause. Some writers have advocated certain "cure-alls," such as the addition to the soil of a single chemical, barnyard manure, or a specific brand of commercial fertilizer. Others assume complete reliance on the correction of soil alkalinity; still others emphasize the necessity of the prevention of excessive early plant growth. Too often, however, such specific recommendations are the result of limited observations or experiments confined to a restricted soil type or locality. Recommendations wherein any single amendment or operation is considered as the only factor which needs be considered by the farmer in the control of late blight and subsequent root rots of the sugar beet must be seriously questioned. It is evident that late blight provides an extremely complex situation and each field, each soil type, and each locality possibly offer a specific problem in control. Especially is this true in such drought years as occurred in Utah in 1919 and 1921.

In view of the facts and observations relating to late blight, particularly in Utah, it would seem that the most hopeful solution lies in the policy of resorting to such cultural practices as will give the young beet an early, vigorous start and guarantee its continued and uninterrupted growth at such a rate as will most adequately bridge the critical period between June and August, during which time beets appear to succumb most readily to late blight. Such practices would involve the factors of soil fertility, soil tilth, adequate cultivation, early planting, and, earlier, lighter, and more frequent irrigation. Any practice that will increase the water-holding capacity of the soil will undoubtedly aid in the solution. Certain features no doubt are to be avoided, particularly delayed irrigation and such practices as would influence adversely the power of the soil to retain its moisture.

Facts indicate that even with the best cultural practices, there is no guarantee that late blight can be completely prevented in epidemic years. Germany and other European countries, with their high cultural and fertility standards attained after years of experience, still face an unsolved late-blight or dry-rot problem. However, much can be done to prevent the serious losses during epidemic years and possibly to eliminate entirely the losses which, through carelessness in cultural practices, mark the more favorable years for sugar-beet growth.

SUMMARY

1. Late blight of the sugar beet is of nonparasitic origin and is characterized primarily by a rather sudden collapse of the leaf tissue. It may not be accompanied by the dry or Phoma rot usually thought to be the characterizing feature of the disease.

2. The root rot that usually accompanies or follows leaf collapse is induced in Utah primarily by *Phoma betae,* which attacks the root only after the resistance of the beet is seriously impaired. Under conditions of continued drought it may not follow leaf blight as an important feature of the trouble.

3. Late blight in Utah is correlated in its occurrence with abnormally low precipitation during June, July, and August. In 1919 and 1921, when the disease appeared in epidemic proportions, the precipitation during these three months was especially low.

4. Experience has shown that late blight might be induced locally any season by culture practices, which results in poor soil tilth, low soil fertility, or such conditions as might seriously disturb the water balance of the plant.

5. Excess alkalinity, high calcium, and high organic content of the soil especially combined with lack of moisture appear as intensifying factors in late-blight production.

6. The exact nature and cause of late blight remain obscure, although the available evidence justifies the conclusion that the disease results in general from nutritional disturbances induced directly or indirectly by an unbalanced water relation of the plant.

7. The control of late blight is a complex problem and methods may vary for each soil type and locality. Under Utah conditions hope lies primarily in so perfecting culture practices as to bridge over the critical period of June, July, and August when the young beets, especially in epidemic years, become definitely weakened and stunted and to create such a condition as will most nearly promote vigorous and uninterrupted growth throughout the entire year.

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THE HOST PLANTS OF THE "BURROWING" NEMATODE, TYLENCHUS SIMILIS[1]

G. H. GODFREY

INTRODUCTION

The so-called burrowing nematode, *Tylenchus similis* Cobb, has been known heretofore as a serious pest only on banana, *Musa sapientum,* and sugar cane, *Saccharum officinarum.* Banana was mentioned as the host plant in Fiji, in connection with the original description of the organism by Cobb (4). This host has since been reported by Illingworth (8) as being seriously affected by *Tylenchus* sp. unidentified in North Queensland, Australia; and by Cobb (5) in Jamaica. In cane the nematode parasite is seriously abundant throughout the Hawaiian Islands (3, 4, 11) and has also been reported (2) from the Philippine Islands, Java, South India, and Formosa. Zimmerman (16) in 1898 described one of the nematodes parasitic on the roots of coffee, *Coffea arabica,* in Java as a new species, *T. acutocaudatus.* From his description and figures it seems clear that his species should correctly be referred to *T. similis,* as has already been suggested by Cassidy (2). The nematode also has been reported by Muir and Henderson (11, p. 245) as occurring on nut grass, *Cyperus rotundus,* in the "nuts" or tubers of which it produces brown lesions. In the course of the present investigation, this host has been observed repeatedly, with striking lesions and an abundance of *T. similis.* The nematode is not sufficiently destructive, however, to be materially helpful in eradicating this troublesome sedge weed. Cassidy and van Zwaluwenburg (3) have reported it on pigeon pea, *Cajanus indicus,* and on the roots of pineapple, *Ananas sativus.* Menzel (10, pp. 18–21) reports *T. similis* on tea, *Thea sinensis,* in the Dutch East Indies. The present paper comments on pineapple infestation and reports two new hosts, edible canna, *Canna edulis,* and sweet potato, *Ipomoea batatas,* with a description of symptoms which are somewhat different from any that have been described in connection with other hosts.

STATUS OF TYLENCHUS SIMILIS WITH REGARD TO PINEAPPLES

The pineapple is probably at most but a minor host of this species. The Nematology Laboratory of the Experiment Station of the Association of Hawaiian Pineapple Canners has never found it in the roots of pineapple plants from the field even though thousands of plants have been examined. Artificial inoculations with nematodes from both edible canna and nut

[1] Technical paper No. 16 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

grass demonstrated the possibility of penetration of roots by the nematodes, but only slight lesions developed and infection did not spread. It is very possible that under field conditions, in pineapple plantings following infested sugar cane, slight infection may occur which probably never persists to a serious degree. Another species of Tylenchus, *T. brachyurus* Godfrey 1929 (7), distinctly different from *T. similis,* is the predominating species found in pineapples and is of distinct economic importance.

OCCURRENCE ON EDIBLE CANNA AND SWEET POTATO

Ripperton (13, 14) has discussed edible canna as being a crop of some commercial promise in these islands. Recently, this crop was studied from the standpoint of nematode resistance, by request of Mr. Ripperton, to determine its possible usefulness as a rotation crop with pineapples. In connection with these studies, lesions on roots and underground stems (corms) were found heavily infested with *T. similis.* Sweet potatoes were found nearby that were likewise infested. Both crops, which were growing only on a small field scale, were in an area that was formerly in sugar cane. It is likely that the cane, a highly susceptible host of this nematode, was the origin of the infestation. Brief mention of this finding was reported early in 1929 in the local organ of the Hawaiian pineapple industry.

Symptoms: The symptoms produced by the nematode in edible canna are characterized by irregularly shaped, dark brown to nearly black spots, varying from a few millimeters to 5 centimeters in diameter and from 2 to 10 millimeters in depth. The surface of spots becomes hard, tough, and leathery, and is somewhat flattened in contrast with the uniformly rounded surface of unaffected areas. In the growing plants it is necessary to tear off leaf sheaths, thus exposing the surface of the corm, in order to distinguish clearly the limits of a lesion. Superficial longitudinal crackings frequently occur through a spot infested with Tylenchus. A cross-section through a lesion shows a collapsed area of dead tissues, black in color and somewhat leathery, underlaid and bordered with finely granular brownish tissues merging gradually into the normal white of healthy tissues. Roots are likewise affected, the symptoms here being dark brown lesions up to a centimeter or more in length, nearly or completely surrounding the rather large roots (3 to 4 mm. in diameter). Roots are easily broken off in the affected region, and cases were seen in which they were rotted completely through. Occasional roots growing on a portion of the corm that has become involved by a large lesion are killed outright. Symptoms on both corms and roots are shown in figure 1.

Symptoms in sweet potato were very similar but not so sharply marked as in the edible canna. The spots observed were smaller and not so deep.

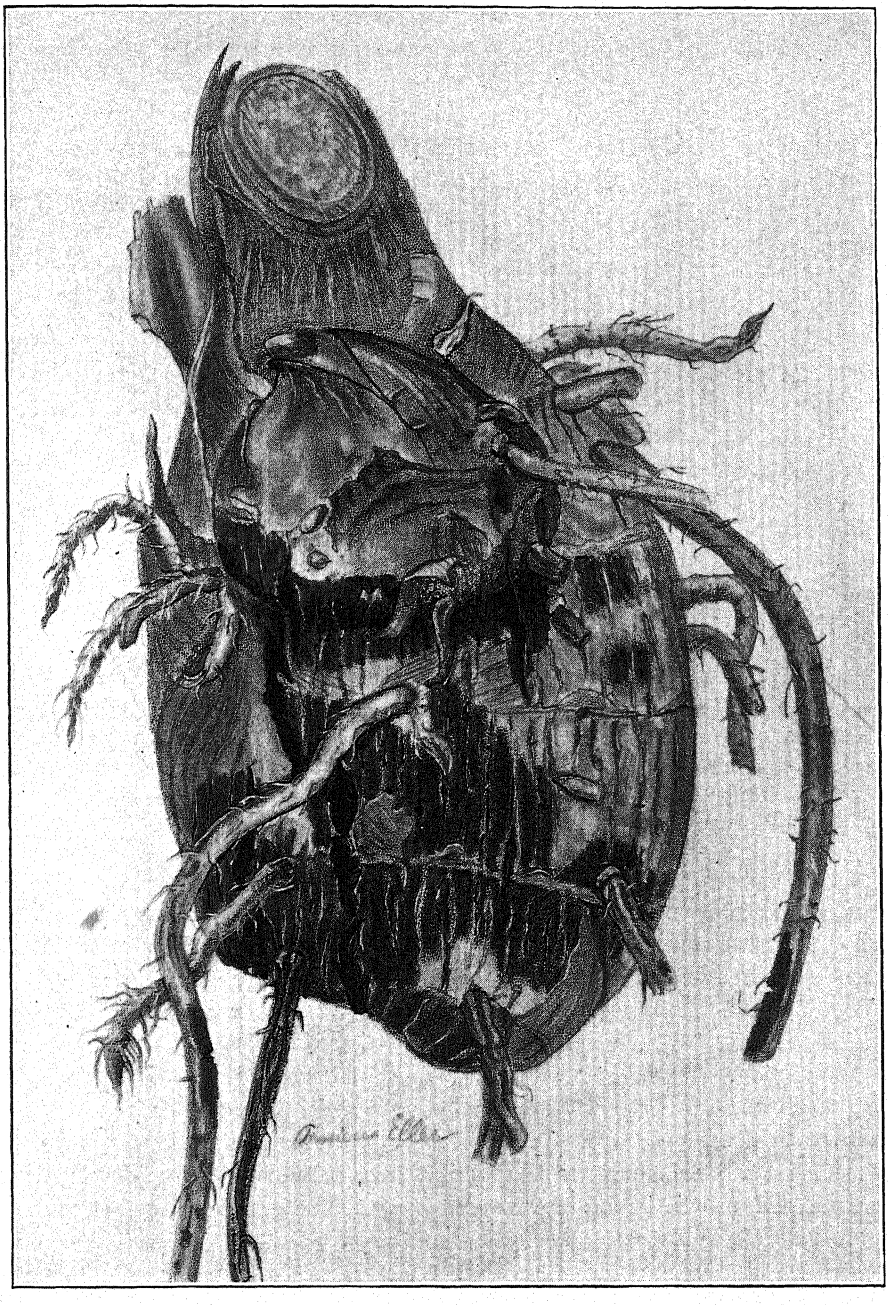


FIG. 1. Symptoms of *Tylenchus similis* in edible canna. Note large dark lesions accompanied by cracking in the corm, and lesions in roots. Note also that one root, issuing from the middle of a large corm lesion, is shriveled and dead. Drawing, about natural size, by Armena Eller.

Heretofore symptoms of disease produced by *T. similis* have been described as occurring only on roots. The finding of seats of infestation in corms and fleshy roots, used as seed material, introduces a new factor of importance into the study of the disease. Incidentally, symptoms on nut grass are very similar to those described for edible canna, except, of course, on a much smaller scale, due to the small size of the nut-grass tubers. Figure 2 illustrates typical symptoms in nut grass.

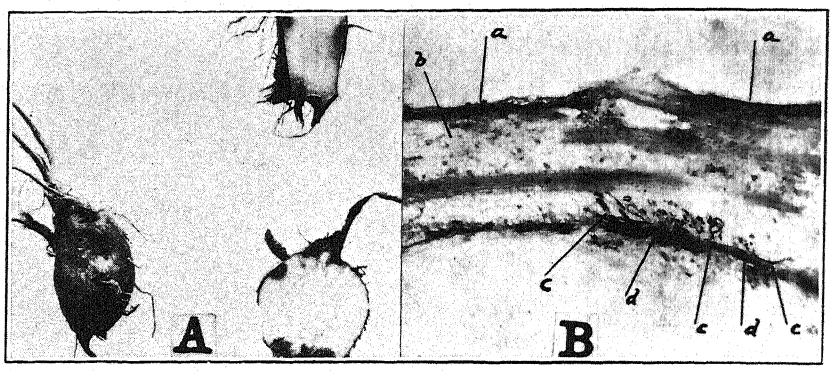


FIG. 2. A, symptoms of *Tylenchus similis* injury in nut grass; surface and sectional views of nut-grass tubers. The discolored regions near the surface are lesions permeated with nematodes. B, *Tylenchus similis* in edible canna. Photomicrograph of hand radial section through corm lesion, the section having been killed in Flemming's solution, dehydrated, and cleared in clove oil; *a*, blackened periderm over lesion; *b*, cortex; *c*, *Tylenchus similis* adults and larvae; and *d*, eggs in the plant tissues. Magnification about 10 diameters.

Economic Importance: Of the edible cannas, more than half of the several dozen random corms examined were infested. A smaller proportion of the sweet potatoes showed symptoms. It is not known how extensively the disease may occur elsewhere than in the particular field on Oahu, where it was found. It is very possible that this was only a chance infestation following infested sugar cane under conditions particularly favorable for transmission. There is reason to believe, however, that this particular sequence would continue to develop the disease in these two crops. Edible cannas grown in pineapple rotations on other Oahu fields showed none of the disease. Corms sent to the station from a large commercial planting at Waimea, on the island of Hawaii, showed some root knot caused by the nematode *Heterodera radicicola* but no *T. similis.*

It would seem that this nematode might become a serious pest in canna or sweet potatoes if culture were continued on the same infested field for some time. A secondary loss might follow from the fact that the presence

of the lesions hastens the deterioration of the corms and roots in storage. A dry rot has been found spreading inward from lesions on materials that were held for some time in the laboratory.

THE CAUSAL ORGANISM

The nematodes are very abundant in diseased tissues of edible canna. Under resting conditions, which seem to occur when the corm is not actively growing, the organisms are frequently crowded together beneath the hardened leathery layer of dead cells which forms below the epidermis. During activity they are found migrating into the deeper tissues and into the advancing edges of lesions. The condition is essentially the same in sweet potato and nut-grass lesions. Living organisms are readily obtained in large numbers by breaking open typical lesions in Syracuse dishes of water. Adult females and males, larvae in all stages of development, and eggs are found together in the host tissues. In a portion of one lesion examined the proportion of adult males to adult females was about one to ten. In another the proportion of males was considerably larger.

A comparison of measurements follows:

MEASUREMENTS OF TYLENCHUS SIMILIS FROM EDIBLE CANNA AND NUT GRASS

Edible canna	*Nut grass*
(Average of 10 adult females)	(Average of 2 adult females)
Decimal formula,	Decimal formula,
2.7 11.6 14.5 55.6 88.8 / 2.3 3.1 3.2 3.5 2.4 — 0.696 mm.	2.6 12.6 15.0 58 89 / 2.6 3.3 3.4 3.8 3.0 — 0.676 mm.
De Man formula, L, 0.696 mm.; a, 28.6; b, 6.9; c, 8.9; V, 55.6 per cent	De Man formula, L, 0.676 mm.; a, 26.2; b, 6.65; c, 9.1; V, 58 per cent
(Average of 8 adult males)	(Average of 4 adult males)
Decimal formula,	Decimal formula,
2.3 11.3 14.7 M 88 / 1.9 2.8 2.9 3.4 2.4 — 0.654 mm.	2.2 12.5 15.0 M 88 / 2.0 2.7 3.0 3.4 2.6 — 0.730 mm.
De Man formula, L, 0.654 mm.; a, 29.4; b, 6.8; c, 8.3	De Man formula, L, 0.730 mm.; a, 29.2; b, 6.7; c, 8.3

These measurements, as well as the detailed analyses, coincide almost exactly with Cobb's description (5), so there can be no doubt as to the correct diagnosis as *T. similis*. Cobb's excellent drawings of both male and female make further drawings of complete anatomical details unnecessary.

HISTOLOGICAL STUDIES

The advance of the nematodes through host tissues appears to be purely mechanical. In histological sections (the materials having been killed

quickly by immersing in killing solutions at about 55° C.), organisms may be seen coiled within single cells whose normal contents have disappeared or they may be outstretched within one or more cells or between cells,

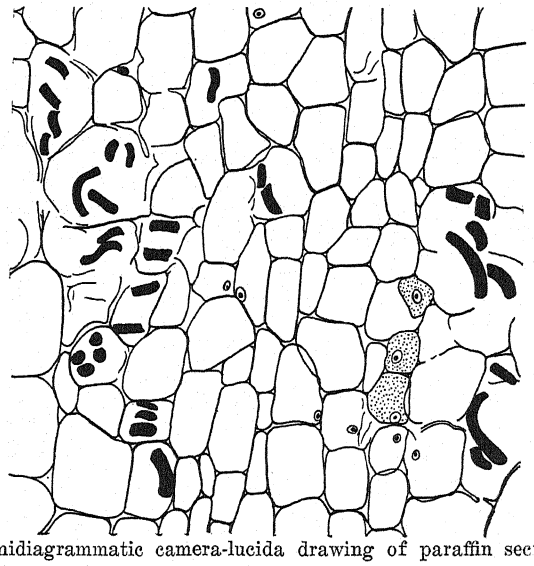


FIG. 3. Semidiagrammatic camera-lucida drawing of paraffin section, 10 μ thick, through border tissue of edible canna-Tylenchus lesion; shows particularly the complete breakdown of cell contents, and the breaking of cell walls, due, apparently, to the mechanical activity as well as the feeding of the invading organisms. Note in one spot apparently uninjured host cells immediately bordering on cells that have been invaded. Magnification about 70 diameters.

promiscuously (Fig. 3). There is evidence that their movements have been more or less violent, as the walls have been broken and displaced. Cases were observed in which a constriction to one-half of the body diameter was evident at the point of penetration through the host-plant cell wall (Fig. 4). This would indicate that considerable pressure had been brought to bear on the walls opposite, in the act of penetration. Many cases, in prepared slides, were observed in which healthy appearing cells, with nuclei intact, immediately bordered upon broken-down cells with nematode traces present. Fungi are frequently found associated with advanced nematode lesions, but they are not invariably present. In none of about twenty slides of rot-free canna corms showing lesions were there any traces of fungi present within the lesion. Figure 2, B, illustrates the location of nematodes in the plant tissues. It is a photomicrograph of a free-hand section about 1 mm. thick, stained and cleared in clove oil, according to Arzberger's method (7).

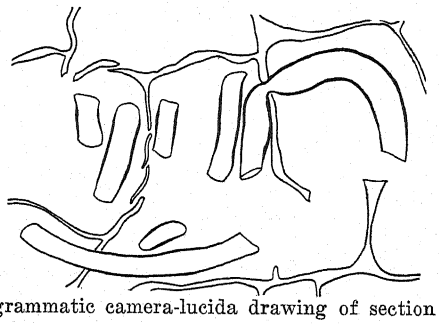


FIG. 4. Semidiagrammatic camera-lucida drawing of section through edible canna lesion, to show constriction in body of nematode as it advances through small hole in cell wall. This would seem to indicate violent activity on the part of the nematode. Magnification about 180 diameters.

SUGGESTED CONTROL

Obviously, diseased canna corms and sweet potatoes used for seed stock provide a sure means of spreading the disease to new localities. If plantings on a large scale are to be made the planting stock should be thoroughly inspected and only seed materials known to be free from the disease should be used.

If the emergency arose, in all probability an effective hot-water treatment could be developed for slightly affected seed corms and sweet potatoes. With similar diseases in other crops in which the infesting nematodes lie relatively near the surface, such treatments have been worked out which kill the nematodes without hurting the "seed." Ramsbottom (12) developed a satisfactory hot-water treatment for the control of *T. dipsaci* in narcissus, and van Slogteren (15), independently, and at about the same time, developed similar treatments for the same nematode in both narcissus and hyacinth. He wrote several papers on the subject only one of which (in English) is here referred to. Byars (1) was successful in killing *Tylenchulus semipenetrans* in citrus nursery stock with hot-water treatments. Godfrey (6) reports successful treatment of the root-knot nematode, *Heterodera radicicola,* in dasheen (*Colocasia antiquorum*), by the same method. Locklin and Newton (9) summarize the recommendations for treatment of narcissus and point out the need for care as to time of application. They likewise give useful citations to literature on the subject.

A further precaution in connection with prospective commercial planting would be the avoidance of lands previously in Tylenchus-infested sugarcane or bananas.

SUMMARY

This paper lists the previous known hosts of the burrowing nematode, *Tylenchus similis,* and adds edible canna, *Canna edulis,* and sweet potato,

Ipomoea batatas, as two new hosts of importance. Symptoms produced on these new hosts are irregularly shaped, dark brown to black spots varying from a few millimeters to 5 centimeters in diameter and from 2 to 10 millimeters deep. Roots are likewise affected, as with the previously reported hosts. The presence of the lesions on corms hastens their decay in storage. Symptoms on nut grass, *Cyperus rotundus,* a previously reported host, are similar to those just described. The writer has never found this species of Tylenchus in pineapple, *Ananas sativus.* Control measures are suggested.

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SOME TECHNIQUE USED IN THE STUDY OF THE ROOT-KNOT NEMATODE, HETERODERA RADICICOLA[1]

G. H. GODFREY[2]

INTRODUCTION

Many investigations (including some by the writer) on the root-knot nematode have been based upon either diseased roots or naturally infested soil as inoculum. In either case life-history stages were not controlled, and results, therefore, were not of so fundamental value as was desired. McClintock (5), working on the effects of various poisons on Heterodera eggs and larvae, developed a fairly satisfactory technique for laboratory-scale investigations. His use of agar media, however, favored the development of bacteria which vitiated his results. Many other workers have, no doubt, used similar methods. In the course of various studies in Hawaii on life history, pathogenicity, environmental relations, and control of the root-knot nematode, it became necessary to regulate the inoculum precisely. It was the aim to obtain in any desired quantity at any time eggs in suitable stages of development or active, vigorous larvae in relatively pure culture. Special technique was therefore developed which made this a simple matter. It is thought that the complete procedure recorded herein may contain enough that is new to make it of value to investigators of the root-knot problem.

SUMMARY OF LIFE HISTORY

To facilitate the clear exposition of this technique, some aspects of the life history of the organism are first briefly summarized. Primary infections are always brought about by larvae which have not grown since hatching. Invasion is always through tender tissues of the plant, usually near the growing point of the root. Once inside the root, the nematode migrates through the cortical tissues and establishes itself in a more or less fixed position, with the body in the cortex and the head upward and inward near the phloem region. Thus the organism lies as a rule, in a diagonal position, with its tail nearest the surface. Figure 1, A, a photomicrograph of an infested tomato root, with nematodes stained and the root cleared in clove oil (method: 3, p. 624) illustrates this relationship. As the nematode grows it becomes greatly enlarged, compressing the plant tissues to some

[1] Technical paper No. 17 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

[2] Grateful acknowledgment is made of credit due Miss Helene T. Morita and Miss Juliette Oliveira for technical assistance in the development of the technique described in this paper.

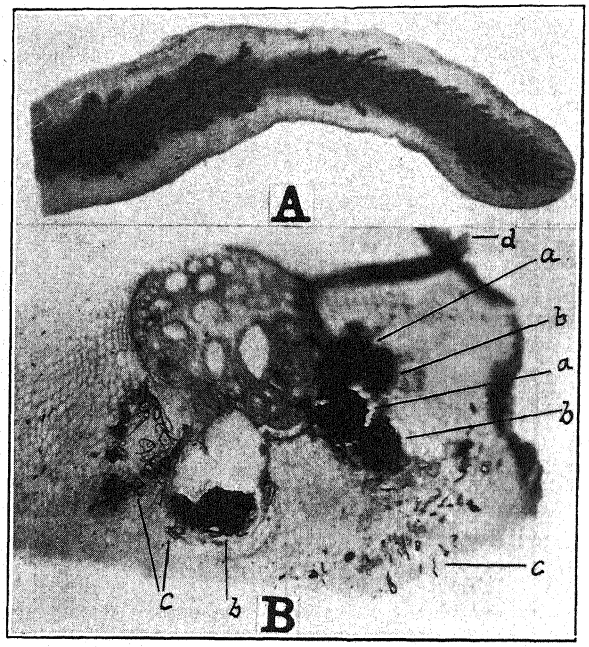


FIG. 1. A. Longitudinal section of primary nematode infestation in tomato root. The section, about 1 mm. thick, was killed in Flemming's solution, dehydrated, and cleared in clove oil. Note the uniform arrangement of the nematodes in the tissues, with the head ends buried in the outer periphery of the stele, and the bodies entirely within the cortex. Magnification about 8 diameters. B. The beginning of secondary nematode infestation in the tomato root. Cross section, about 1 mm. thick, of a tomato root gall, cleared as described in the text, showing—*a*, body of old female, no longer living at time of fixation; *b*, egg masses, where originally deposited; *c*, migrating larvae, recently hatched from egg masses; *d*, small branch rootlet. Magnification about 15 diameters.

extent, and its caudal end approaches the surface. As the first macroscopic sign of maturity, the by this time almost spherical female extrudes a gelatinous substance from the vulva. Very soon after, eggs appear, embedded in the center of the gelatinous matrix. Eggs continue to be extruded by the female until eventually the gelatinous "egg-mass" or ootheca is as large as or larger than the body of the female from which it came. If development has been rapid, the developing egg mass often bursts through the side of the root and appears as a yellowish semitransparent body closely attached to the root. Often under favorable conditions, the ground having been heavily infested and temperature favorable, such egg masses attached to roots can be seen in great numbers on a single plant. (Fig. 2.)

The time when this stage is reached is an important consideration. Temperature is an important factor in this. Tyler (7), in a preliminary

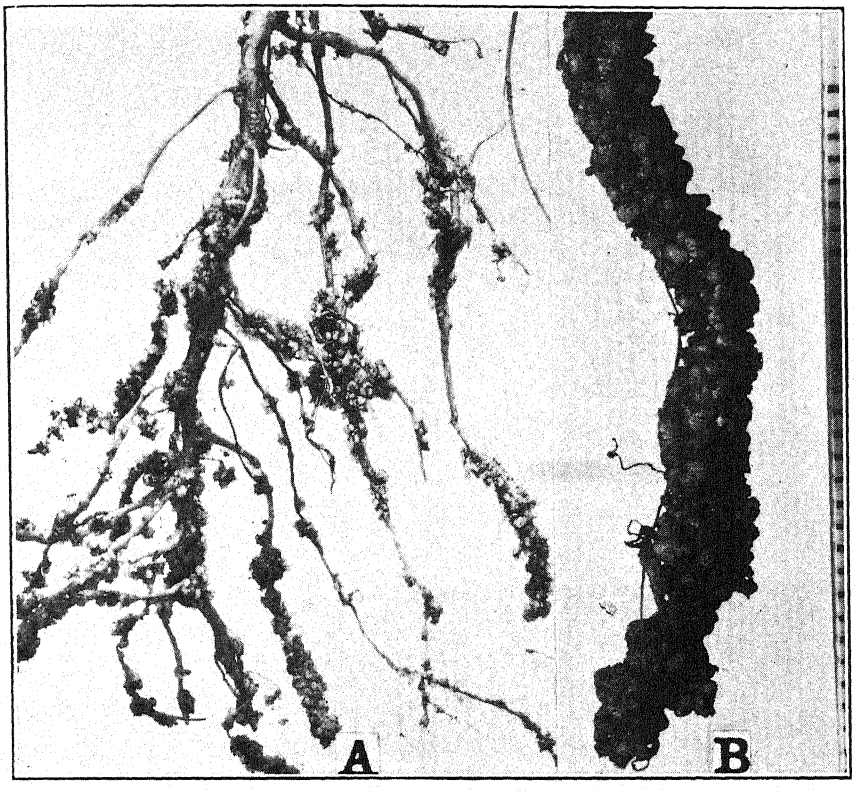


FIG. 2. A. Cowpea root heavily infested with the root-knot nematode, showing abundant protruding egg masses. About natural size. B. Individual cowpea root magnified to show the nature and abundance of the protruding egg masses. Scale in millimeters.

report, promises some exact information on the influence of temperature on length of life history. Within the range of tolerance of the nematode, the length of life history (from first penetration of the host plant root to maturity) shortens as the temperature increases. Under average crop conditions, where nematodes occur, first-egg production may be expected in from 20 to 30 days, depending upon soil temperature. With favorable conditions, egg deposition continues 10 days or longer.

Temperature has another important effect in connection with our technique. It influences the type of root growth, the resultant of host-parasite relations. This was demonstrated by the writer's earlier study of temperature in relation to root knot (2). In figures 8 and 9A, that paper, are illustrated tobacco roots grown in infested soil at 25° C. with egg masses protruding in abundance, and another root grown at a lower temperature

(19° C.) in which larger galls developed and no egg masses were evident on the surface. Under the latter conditions, eggs are produced within the root, a condition not desirable from the point of view of our technique. Probably the case recorded by Steiner (6) of superficial appearance of the root-knot nematode on the roots of freesias, and other similar observations, are explainable in part, on the basis of temperature. I have seen numerous instances of similar development of root knot on a wide range of cultivated plants and weeds growing in warm soil.

Internal egg masses are likewise produced in the case of secondary infections. Larvae arising from first-generation eggs sometimes migrate through the host tissues to another location within the same root, where they establish themselves and develop normally. Figure 1, B, illustrates such migration of the second generation, in tomato roots. Such is the material an investigator deals with when he uses roots with irregular large galls (2, p. 226) as source of inoculum. Nematodes in all stages of development are always to be found, and they are released from the plant tissues only gradually and through a long period.

OBTAINING EGG MASSES IN QUANTITY

After the foregoing discussion, it is evident that it is possible to attain the desired goal, viz, an abundance of superficial egg masses at any specified time, by judicious regulation of conditions. As the first steps in our technique then, (1) pots, flats, or isolated plots of good friable soil are prepared, the soil temperature to be 22° C. or higher; (2) seeds of a suitable susceptible host plant (we have used Whippoorwill and Groit cowpea *Vigna sinensis,* to good advantage) are planted; (3) the soil around the plants is inoculated heavily at sprouting time with *H. radicicola* larvae (method for obtaining which follows); (4) good growing conditions are maintained.

By daily removal of a plant or two and examination of roots, it is possible to obtain organisms in abundance in any desired stage of development. Males are obtained in great numbers by washing the carefully removed roots, a day or two prior to maturity of the females, and screening the washings from the roots through a 100-mesh or finer sieve. Nonsegmented eggs in quantities are obtained by dissecting egg masses the day they first become evident or on the day after. Examination of roots for this purpose begins on about the twentieth day or earlier, if the soil is unusually warm. Later examinations, up to the 35th day, or even later, usually disclose an abundance of roots covered with egg masses. In this instance, one piece of root about an inch long, shown in figure 2, B, had about 200 egg masses attached. A single plant, such as that shown in figure 2, at this rate had approximately 4,000 egg masses attached, each containing between 500 and

1,000 eggs. (As many as 1,300 were found in one egg mass in this laboratory.) This offered potentialities for between 2 and 4 million new infections!

Egg masses a week or more old invariably contain eggs in different stages, varying from nonsegmented to fully developed larvae, and usually many newly hatched larvae as well. A continuous supply of such egg-mass material is maintained by planting a succession of cowpeas at intervals of two weeks or thereabouts in a plot of ground set aside for this special purpose.

TECHNIQUE

Eggs: If one wishes to work with eggs, for chemical or environmental studies, it is possible to select them and pipette them out from a Syracuse dish of water resting on the stage of a binocular dissecting microscope. A fine-drawn capillary pipette, either with a rubber bulb or with a thistle-tube head covered with a thin rubber membrane, is necessary for this. By first drawing in only a little water, individual eggs, dissected out with needles, are lifted and mounted at will. For more extensive studies, egg masses in which the eggs are protected to a degree by the thick gelatinous matrix are readily removed and handled individually or by hundreds or thousands.

Larvae: A study of the larvae demands the incubation of eggs under conditions favorable for hatching and for maintenance of vitality in the newly hatched larvae. For small-scale studies egg masses, washed in a few changes of water or even surface-sterilized, are mounted in hanging drops of water or placed in small Syracuse dishes and left in a moist chamber to prevent evaporation. Aeration is necessary, for eggs will not hatch without sufficient oxygen. Hatching starts in from a few hours to several days, depending upon the stage of maturity of the contained eggs and upon temperature. Any temperature between 24° and 28° C. is favorable for rapid hatching. It is necessary to change daily the water surrounding an egg mass to avoid development and accumulation of putrefying bacteria. When this is done, 100 per cent hatch from an egg mass is often obtained. Hatched larvae, removed with the water, are placed in a small beaker in the ice box, at 8 to 12° C., and the daily accretions added thereto. Calculations of total hatch are made by counting the daily hatch as it is removed. Usually not more than 10 days or two weeks are required for the practically complete hatching of eggs in an egg mass. The product of one or two or of many egg masses is thus obtained for experimental work.

Very much more extensive work with newly hatched larvae numbering millions can be accomplished by altering the method of hatching egg masses. This has been accomplished in the writer's laboratory by select-

ing a large number of roots with the egg masses well developed, washing them gently, to free them from excessive adhering soil and contaminating nematodes, and placing them on woven-wire screens in covered dishes containing a small quantity of water. The water must be just sufficient to barely reach the screen and keep the roots moist by capillarity. The larvae then hatch, migrate to the screen and thence to the water. With the right kind of root materials a tremendous "hatch" is often obtained in a very short time. In an overnight period the writer has often obtained a suspension of larvae sufficient to make it distinctly milky in appearance. By setting the same roots several times subsequently, each time over fresh water, additional large quantities of larvae are obtained. This process is suggestive of the well-known Baermann method (4, pp. 90–92) of isolating animal-parasitic nematodes (funnel with rubber bulb and clamp), which method can, indeed, be used at this stage.

A suspension in a beaker or a tall cylinder of water may be concentrated by allowing it to stand an hour or more, sufficient time to permit the larvae to settle to the bottom, and then decanting. The larvae are so small and light that they stay in relatively uniform suspension for several seconds after the suspension is thoroughly roiled. The numbers in aliquot parts of such suspensions are counted according to the methods described in detail by Cobb (1). This gives a basis for determining volume and concentration of the suspension to use in order to obtain approximately the numbers of larvae desired for specific operations.

DISCUSSION

By these methods, then, Heterodera material is readily made available in various stages of development and in any desired quantity, for research work. By way of comparison with these controllable stages, infested roots containing eggs and larvae may be used in parallel experiments. With such roots the organisms are protected from immediate contact with the external environment and are therefore more resistant to unfavorable soil conditions and to specific control measures. Studies of the organisms in an infested garden or field must take this into consideration.

SUMMARY

This paper details specific methods used to obtain for experimental purposes an abundant supply of egg masses and larvae of the root-knot nematode, *H. radicicola*. To clarify the exposition of this technique, the life history of the organism is briefly reviewed, special emphasis being placed on the influence of temperature on rate and manner of development. Root-knot-susceptible cowpeas grown in warm, heavily infested soil, develop an

abundance of superficial egg masses. These are removed and "incubated" separately on slides or in Syracuse dishes; thus freshly hatched larvae suitable for laboratory studies are obtained. For large-scale operations, roots containing egg masses in abundance are incubated in bulk and larvae are obtained by the millions.

EXPERIMENT STATION, ASSOCIATION OF
HAWAIIAN PINEAPPLE CANNERS,
HONOLULU, T. H.

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"BUCKSKIN," A DESTRUCTIVE GRAFT-INFECTIOUS DISEASE OF THE CHERRY

T. E. Rawlins and W. T. Horne[1]

A disease of the sweet cherry has been causing much loss for some years in some of the cherry-growing districts of northern California. The disease has been observed on Napoleon, Bing, Black Tartarian, Chapman, Oregon, and Rockport varieties. It has not yet been observed on a variety known locally as "Long Stem Bing."

The fruits show the most conspicuous symptoms of the disease, fruits on infected portions of a tree being more or less conical in form and failing to mature. Just prior to ripening, the diseased fruits cease to develop and the surface of the blossom end takes on a dull "buckskin" appearance. Such fruits are shown at A in figure 1. The fruits remain in this condition for several weeks after healthy fruits have ripened. In the Napoleon variety the diseased fruit fails to take on the red cheeks characteristic of this variety and remains yellowish to green and shows occasional brownish depressions toward the blossom end. The darker varieties, such as Chapman, Black Tartarian, and Bing, remain red when infected and do not darken until after shriveling. Diseased fruits hang on the tree for some time and finally become badly shriveled as shown at B in figure 1. The pedicels of diseased fruits are usually shorter than those of normal fruits.

Diseased fruit is usually first found on a single limb or several adjacent limbs in a tree. In other cases the diseased fruit may be scattered through

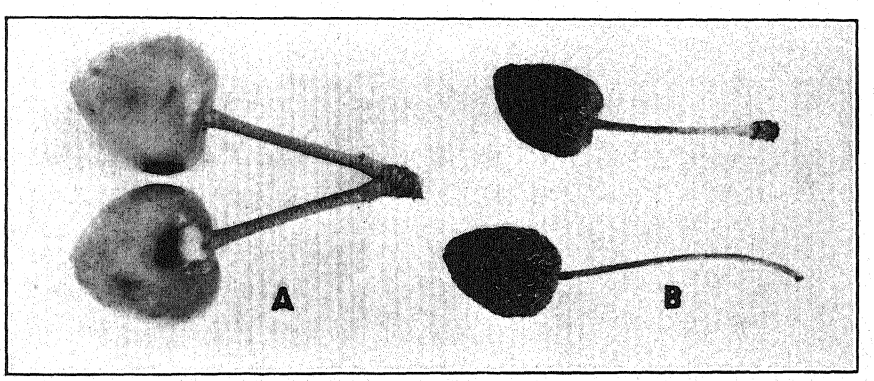


Fig. 1. Diseased Napoleon fruits. A. An early stage of the disease. B. Diseased fruits which have hung on the tree for about a month after the picking season.

[1] The writers wish to express their appreciation of the work of Mr. A. Morse, who assisted in the grafting experiments, and of the generosity of Mr. Sidney Jones, who donated a plot for experimental work.

the tree. In certain cases single spurs have been observed to bear some healthy fruit and some diseased fruit. After a tree has been infected for several years most of its fruit is usually worthless.

During early autumn the leaves on infected portions of the tree show a peculiar orange to maroon coloration along the base of the midrib and extending out along the basal lateral veins. This is shown in figure 2, the light-appearing portions seen along the base of the midribs and basal lateral veins in the illustration being maroon in the subject. This symptom varies considerably according to the season. During the 1929 season this symptom was less conspicuous than during previous seasons.

Diseased trees show considerable variation in vigor. Certain infected trees may appear quite vigorous for some years, while others may show lit-

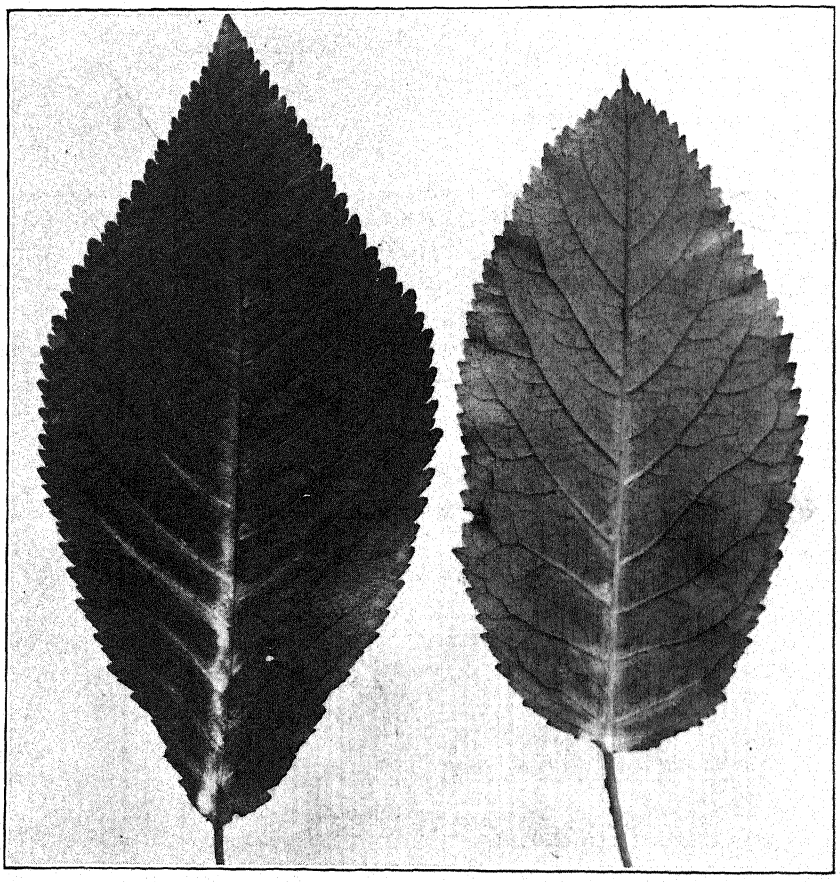


FIG. 2. Showing the peculiar coloration which occurs along the base of the midrib and basal lateral veins of diseased leaves during early autumn.

tle growth and may have small leaves or dead limbs. The Napoleon trees appear to be most severely injured by the disease. Infected trees of this variety often have dead limbs and in light soils the trees may die within a few years.

After the disease has invaded an orchard it usually spreads rather rapidly. A rough survey during 1929 indicated that in one orchard about 10 per cent of the trees had been infected during that season. At the present time 50 per cent or more of the trees are infected in certain orchards. Most of the infection appears to come from trees within a radius of about 50 yards. Some orchards, approximately a quarter of a mile from severely infected orchards, have remained uninfected.

EXPERIMENTAL

Although the microscopic and culture studies which have been made on infected tissues are perhaps not so thorough as might be desired, all such studies have failed to show the presence of a visible pathogene.

During the last several years numerous attempts have been made to transmit the disease by grafting scions from diseased trees into healthy young trees. In some cases the diseased scions carried flower buds and therefore produced fruit the same season that they were set into the healthy trees. In order to hasten fruit production healthy scions bearing flower buds also were grafted into healthy limbs of some of the young trees in which diseased scions had been grafted. Similar healthy scions were also grafted into noninoculated check trees. The above procedure is very satisfactory in that it permits one to determine the transmissibility of the disease without waiting several seasons for the production of fruit by young trees.

In other trees the diseased scions were set in 1928 and the young trees began to bear fruit along the trunk and in certain branches in 1930.

All of the results have been condensed in table 1. A study of this table shows that the disease was transmitted to the healthy trunk and to healthy scions in a large proportion of the trees in which diseased Napoleon scions had been grafted. Of 27 trees in which diseased Napoleon scions were grafted 12 showed distinct symptoms of the disease in the fruit on either the healthy scion or the trunk, 6 showed slight symptoms of disease, and 7 appeared to be healthy. All diseased Napoleon scions which bore fruit produced diseased fruit.

Diseased Black Tartarian scions behaved very differently. All of the 3 diseased Tartarian scions which bore fruit produced healthy fruit and in only one case was there any evidence that the disease was transmitted by a diseased Tartarian scion. Strangely, no difficulty was experienced in

TABLE 1.—*Transmissibility of buckskin disease by grafting*

No. of trees	Stock	Trunk	Diseased scion	Healthy scion	No. of trees bearing fruit on diseased scion	Condition of fruit on diseased scion	No. of trees bearing fruit on healthy scion	Condition of fruit on healthy scion	No. of trees bearing fruit on trunks	Condition of fruit on trunks
13	Mazzard	Napoleon	Napoleon	Napoleon	6	DDDDDD	12	DHDDD? ?HD?H?	0	
1	,,	,,	,,	None	1	D			0	
7	,,	Tartarian	,,	,,	0				7	HDD?HDD
2	,,	,,	,,	Tartarian	0		2	DH	0	
1	,,	Long Stem Bing	,,	,,	0		1	H	0	
1	,,	Deacon	,,	,,	0		1	?	0	
1	,,	Schmidt	,,	,,	0		1	D	0	
1	,,	Napoleon	,,	Windsor	0		1	D	0	
2	,,	,,	Tartarian	None	1	H			1	H
1	,,	,,	,,	Schmidt	1	H	1	H	0	
1	,,	Unknown	,,	Probably Schmidt	0		1	H	0	
2	,,	Tartarian	,,	None	1	H			2	H?
6	,,	Napoleon	None	Napoleon			6	HHHHHH	0	
3	,,	,,	,,	Tartarian			3	HHH	0	
12	,,	Tartarian	,,	None					12	HHHHHH
5	,,	Napoleon	,,	,,	0				5	HHHHHH

?—Fruit shows some symptoms of disease but symptoms are insufficient to be sure that it is diseased.
D—Fruit diseased.
H—Fruit healthy.

transmitting the disease from diseased Napoleon scions to healthy Tartarian trunks and healthy Tartarian scions.

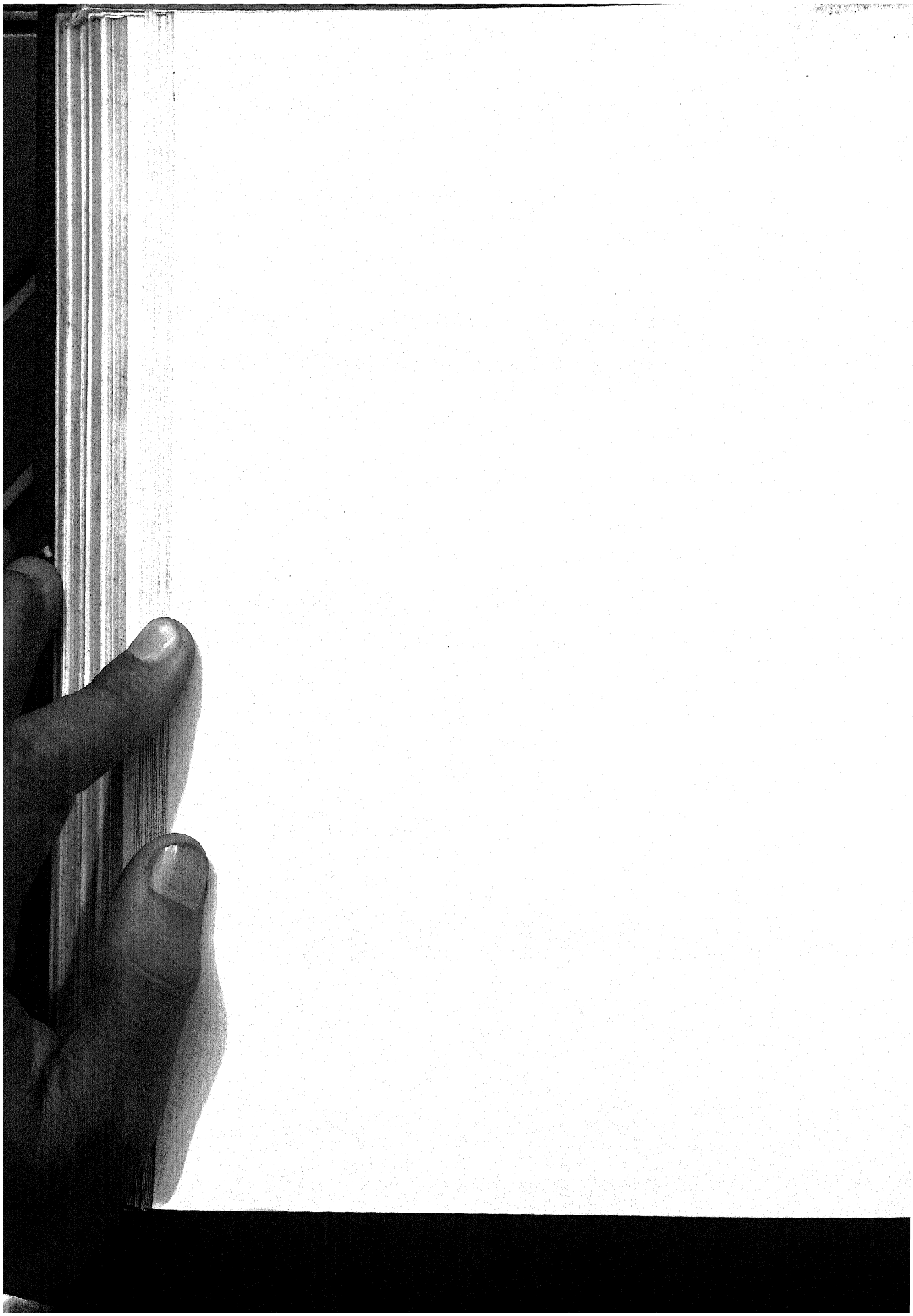
None of the 26 check trees in which no diseased scions were placed showed symptoms of the disease.

A record was kept of the distance between the diseased scion and the healthy scion on each tree. It was found that the greatest distance between such scions in cases where the disease was transmitted was 2 feet, being 1 foot down from the diseased scion to the trunk and 1 foot out from the trunk on another limb to the healthy scion. The time between setting the scions and the observation of diseased fruit on the healthy scion was 4 months. Our experiments in this field were not sufficient to draw any conclusions as to what the maximum rate of movement or the mean rate of movement of the causal agent may be. The experiments also were insufficient to indicate whether the observed movement was that of a causal agent or that of the products of a causal agent. The fact that growers have been unable to check the progress of the disease in a tree by cutting well below any diseased fruit would indicate that the causal agent moves even more rapidly than the symptoms.

SUMMARY

A destructive disease of the sweet cherry is described. This disease may be transmitted to healthy trees by grafting scions from diseased Napoleon trees into healthy trees.

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PHYTOPATHOLOGICAL NOTES

Physiologic Specialization in Sclerospora graminicola. Experiments were conducted in which various species of the grass family were exposed to infection from oospores of *Sclerospora graminicola* (Sacc.) Schroet. The oospores were obtained from *Setaria viridis* (L.) Beauv., *S. magna* Griseb., *S. italica* (L.) Beauv., and *Pennisetum typhoideum* Rich. The results are summarized in table 1.

TABLE 1.—*Results obtained from exposing four species of Poaceae to infection by oospores of Sclerospora graminicola collected from four of its hosts*

Species exposed to infection	Per cent plants infected[a]			
	Oospores from			
	Setaria viridis	*S. magna*	*S. italica*	*Pennisetum typhoideum*
Setaria italica	56	76	46	0
S. magna	41	45	15	0
Euchlaena mexicana	71	40	14	0
Pennisetum typhoideum	0	0	0	63

[a] The total number of plants exposed to infection varied between 28 and 328 in each case.

Oospores collected from three species of Setaria produced abundant infection on *S. italica, S. magna,* and *Euchlaena mexicana* Schrad. Also, the infection of *Pennisetum typhoideum* was obtained from oospores taken from *P. typhoideum.* But oospores from the three *Setaria* spp. failed invariably to produce infection on *P. typhoideum* and *vice versa.* This supports the observation of Weston and Weber[1] that the downy mildew on *S. magna* does not pass to *P. typhoideum* under field conditions in Florida. Butler[2] also has reported that the fungus on *S. italica* did not pass to *P. typhoideum,* grown in the vicinity of Pusa.

These observations and the experimental data suggest the presence of at least two physiologic forms of *Sclerospora graminicola.* One form will attack *P. typhoideum* only, and the other form can infect *Setaria viridis, S. italica, S. magna,* and *E. mexicana.*

[1] Weston, W. H., Jr., and G. F. Weber. Downy mildew (*Sclerospora graminicola*) on Everglade millet in Florida. Jour. Agr. Res. 36: 935–963. 1928.

[2] Butler, E. J. Fungi and disease in plants. 547 pp. Thacker, Spink & Co. Calcutta and Simla. 1918.

None of the collections of oospores of either physiologic form caused infection on *Panicum miliaceum* L. or on *S. glauca* (L.) Beauv., although many plants were exposed in each case. This is partly confirmed by the work of Melhus *et al.*[3]—B. N. UPPAL and M. K. DESAI, *College of Agriculture, Poona, India.*

Bacteria producing rot of apple in association with the apple maggot, Rhagoletis pomonella. In making a study of the infestation and injury to apples by the apple maggot or "railroad worm," *Rhagoletis pomonella* Walsh, it has been frequently noticed that in softer varieties of apples a characteristic decay accompanies the work by the larva of this insect. Such a condition has not been normally found in apples attacked by other insects. Therefore, the question has arisen whether a pathogenic organism is associated with the apple maggot and whether the organism is responsible for rot which commonly occurs in maggot-infested apples after they have been placed in storage. With this in mind, a preliminary study was made with infested apples from several orchard districts of Wisconsin to determine the relation between the rot, the apple maggot, and associated microorganisms.

From marginal limits of larval burrows in Wealthy apples, several cultures possessing different characters were isolated in 1929, but only one culture, which repeatedly appeared pink, produced rot when inoculated upon sterile apple plugs and entire apples. In purifying this pink culture it was found to contain both bacteria and yeast cells. These were readily separated, and further inoculations with them upon sterile apple tissues showed that the bacteria and the combination of bacteria and yeast produced a rot but the yeast alone gave negative results.

This same type of bacterium was again isolated from larval burrows and from the adult apple-maggot fly in the fall of 1930 at Gays Mills, Wisconsin, where studies of the apple maggot were being made. As each of three strains thus far studied consistently developed rot in six different varieties of apples, it is evident that this organism is capable of producing decay of apple tissue. It is probably distributed by the adult fly and spread by the larvae. More extensive studies are now under way on the pathogenicity and bacteriological characters of the organism.—T. C. ALLEN, Department of Economic Entomology, University of Wisconsin, Madison, Wis.

A method of detecting and demonstrating early leaf infections of apple scab. During the first few days after apple-scab infections appear on the

[3] Melhus, I. E., F. H. Van Haltern, and D. E. Bliss. A study of *Sclerospora graminicola* (Sacc.) Schroet. on *Setaria viridis* (L.) Beauv. and *Zea mays* L. Iowa Agr. Exp. Sta. Res. Bul. 111: 297–338. 1928.

foliage it is somewhat slow and laborious for even the experienced worker to note all of the lesions and practically impossible to demonstrate their presence to the average fruit grower. It occurred to the writer that cobalt paper, originally used by Stahl in 1894 and now commonly employed by plant physiologists in studies of transpiration, might be used. Filter paper was soaked in a 5 per cent solution of cobaltous chloride and dried in the oven. A small press was made by fastening two lantern-slide cover glasses with a flexible hinge and attaching several layers of blotter paper to the inside of one of the slips with passe-partout strips. An apple leaf was placed on the blotter paper, a sheet of the dry cobalt paper placed over the upper surface of the leaf, and the press closed and held with a pair of rubber bands. In a few seconds the blue paper turns white over the area of the scab lesions, due to the increased water loss in the affected areas, giving a very striking print of the amount of scab present. By outlining the white spots on the paper a permanent record may be made. Prints were made also from leaves still attached to the tree, making a series of prints of the same leaves possible.

For use in the field a small tin box was fitted with a container of calcium chloride, so that a supply of the cobalt papers could be kept dry. Sheets of the cobalt paper were used over and over in the field, being placed on hot metal of the automobile to dry the paper between tests.—W. D. MILLS, Department of Plant Pathology, Cornell University, Ithaca, New York.



BOOK REVIEWS

Pathologie des Protoplasmas. By Ernst Küster. 200 pp., 36 figures. 1929. Gebrüder Borntraeger, Berlin.

The present volume is submitted as Part I of a more inclusive work, "Pathologie der Pflanzenzelle," announced as Volume III of a series of monographs on protoplasm completed or in preparation by authorities in various lands, the titles made known thus far appearing either in English, German, or French. Like the treatise on pathological plant anatomy by the same author, the several editions of which have served widely and well for more than two decades, the book presents a large mass of information gathered from numerous scattered sources. The text is divided into two chapters; the first, "Changes in Form," includes under separate headings discussions on plasmolyses, experimental shaping of protoplasts, divisions of protoplasts, protoplasmic deposits, plasmoptysis and related phenomena, local necrosis, and increase in size of uncovered protoplasts by swelling. In the second chapter, "Changes in Structure," are included sections dealing with changes in layered structure of protoplasm, coagulation of protoplasm, vacuolate or foamy degeneration, and swelling of protoplasm.

As the term "protoplasm" is used throughout the book in the sense in which it was early employed by Mohl, referring therefore to the material later designated as cytoplasm, any discussion of the nucleus or of chromatophores, except in occasional passages, is excluded. Yet, the variety of phenomena that comes in for attention is a surprisingly large one, and the student who can read any considerable portion of the text without uncovering some reference or other to some manifestation of protoplasm which he had not suspected was recorded in the literature is either unusually well informed or has not used his microscope to good purpose. I experienced an agreeable surprise, for example, on reading the section on "Plasmoptysis and Related Phenomena" to discover the wealth of observations extant concerning the expulsion of protoplasm from cells provided with walls, instances of which had frequently come to my notice in examining cultures of phycomycetous fungi. It is to be regretted that at times owing to the plethora of material available for discussion—the bibliography occupying 28 well-filled pages—the author was constrained often to summarize more rigorously than might be expected in a monographic treatment. This brevity, however, together with the commendable avoidance by the publisher of wastefully wide margins, disagreeably thick paper, and unnecessarily large print, so often employed to augment bulk and price, has resulted in a compact volume at once convenient to use and not excessively expensive.—CHARLES DRECHSLER, Bureau of Plant Industry, Washington, D. C.

Phytopathologische Zeitschrift. Edited by E. Schaffnit in collaboration with Appel (Berlin-Dahlem), Brierley (Harpenden), Foëx (Paris), Gassner (Braunschweig), Gäumann (Zurich), Jaczewski (Leningrad), Klebahn (Hamburg), Liro (Helsinki), Müller (Angora), Naumov (Leningrad), Petri (Rome), Hemmi (Kyoto), and Westerdijk (Baarn); 1 to 2 volumes appearing each year, each volume consisting of 6 issues; published by Paul Parey, Berlin. 1929—.

The "Phytopathologische Zeitschrift" represents a continuation of the "Forschungen auf dem Gebiet der Pflanzenkrankheiten und der Immunität im Pflanzenreich," brought to a close with the fifth issue at the end of the year 1928. In the publisher's announcement the new periodical is described as resting upon an international foundation—and this description has been substantiated tolerably well by the first volume, which appeared in 1929, as well as the several parts of the second volume already published. For, although of the twenty-three papers contained in the first volume, only two are in French and only one is English, the disproportion in favor of German is pronounced more in respect to language than to source, inasmuch as of the twenty remaining papers not more than eleven are referable to German laboratories, four of the other nine written in German having had their origin in Russian, two in Dutch, two in Swiss, and one in Italian establishments. The German displayed in the articles contributed from outside the German-speaking regions shows little or nothing in the way of obviously alien peculiarities. However English-speaking readers may perchance find some refreshing enjoyment in the novel though sometimes fetching expressions to be encountered in the one article appearing in their language.

For the most part the papers in the first volume of the "Zeitschrift" are expressive of the more intensive type of investigation which, especially during the last two decades, has provided increase both in fundamental knowledge and in economic mastery. Among subjects dealt with may be mentioned, for example, the effect of carbon dioxide on development of rust, the breeding of wheat for resistance to stripe rust, the varietal response of wheat and oats to stripe rust and loose smut, respectively, the physiologic races of stem rust, the influence of fertilizers on the susceptibility of plants to disease, the pathological effect of virus diseases on cell structure, and the biochemistry of potato-tuber rot caused by late blight. Yet, the older aspects of plant pathology have not been neglected. At least three papers are devoted primarily to the description of various parasitic fungi, one paper deals at some length with the phylogeny of fungi, another is devoted to the description of a new bacterial disease of chicory and the organism responsible for it, and still another discusses the bacteria associated with clubroot.

The new journal is put up in excellent form, the paper being of good quality, the type clear, and the margins sufficiently but not unnecessarily wide. The half-tone reproductions of photographic originals, as well as the colored plates, are of uniformly high merit. The drawings are always well reproduced, whether, as fortunately must often have been the case, the originals were carefully executed, or whether, as evidently was true in other instances, the somewhat cavalier draftsmanship of the contributor can hardly have promised an edifying end product. All in all, the periodical well deserves the place in the front rank of scientific publications which apparently it already occupies. That it will be found in all libraries serving the needs of plant pathologists may be taken for granted. I predict, however, that copies in lending libraries will be out much too frequently to be immediately available and that the active worker will find such measure of comfort in having a complete set on his own shelf as will repay him many times over for the outlay of the rather high, though according to European standards, not excessive price of subscription.—CHARLES DRECHSLER, Bureau of Plant Industry, Washington, D. C.



EMIL GODFRED ARZBERGER

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EMIL GODFRED ARZBERGER
1877–1930

A. B. STOUT

Emil Godfred Arzberger was born at Helenville, Wisconsin, on October 3, 1877. He died from endocarditis at Clarendon, Virginia, on January 29, 1930. His body rests in the cemetery at Helenville, Wisconsin, close to the scenes of his childhood. Of the immediate family a father, three brothers, and four sisters are still living.

For his early education Mr. Arzberger attended the public schools with graduation from the High School of Jefferson, Wisconsin, in 1897. He attended the State Normal School at Whitewater, Wisconsin, graduating there in 1903, after which he was principal of a graded school in Wisconsin for two years. He then entered the University of Wisconsin, completed the work for the degree of Ph.B. in 1906, and remained for three years as graduate student and assistant in botany. During the academic year of 1909–10 he was a Fellow at the Missouri Botanical Garden in St. Louis, Missouri, and at the end of this year he received the degree of M.A. from Washington University. The thesis for this degree was entitled "The fungous root-tubercles of *Ceanothus americanus, Elaeagnus argentea,* and *Myrica cerifera,*" and it was published in the Twenty-first Annual Report of the Missouri Botanical Garden. From 1910 until 1913 he was assistant botanist of the Ohio State Experiment Station with principal work in pathology. For several months during the summer of 1913 he held a Research Scholarship at The New York Botanical Garden and from this he went to the Office of Nematology as assistant pathologist, and this position he held until his death.

Mr. Arzberger's interests in botany centered in pathology and mycology. Most of his research was devoted to organisms that inhabit the roots of plants and to mycorrhizae. He obtained in pure cultures various of the fungi concerned in the formation of mycorrhizae, he made careful cytological studies of these structures, and he was concerned in determining the relation of their presence to the growth which plants made. He was widely acquainted with the literature in this field and was able and critical in the consideration of it.

By natural inclination, Mr. Arzberger was of a quiet and retiring disposition. His friendships were deep and lasting. Those who knew him recognized and admired his integrity, sincerity, and worth, and were impressed with his knowledge, skill, and ability in the field of botanical science which he loved.

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PRELIMINARY EXPERIMENTS ON THE CONTROL OF CEREAL RUSTS BY KOLO DUST[1] [2]

W. C. BROADFOOT[3]

INTRODUCTION

In 1924 Kightlinger (5) reported "phenomenal control" of rusts on oats in New York by dusting with sulphur. Since that time considerable emphasis has been placed on and impetus given to sulphur dusting as a method for the control of cereal rusts. In 1926 Kightlinger and Whetzel (6) in New York, Bailey and Greaney (1) in Manitoba, and Lambert and Stakman (7) in Minnesota obtained evidence in support of Kightlinger's earlier statement that sulphur controls rust under certain conditions. In 1927 Bailey and Greaney (2) reported the results of trials with horse-drawn traction dusters as well as those of small-plot experiments. They stated, however, that stem rust was such a negligible factor in Manitoba that year that conditions did not favor testing the effectiveness of sulphur dust under field conditions. Bailey and Greaney (3), in 1928, reported favorable results in some instances from dusting under field conditions both by horse-drawn dusters and by aeroplane. Greaney (4) also reported that the fineness of the particles, humidity, and temperature greatly influenced the toxicity and fungicidal effectiveness of the dust.

Preliminary experiments also had been made in Minnesota. It had been found by Lambert and Stakman (7) that Kolo dust, as well as certain other dusts, would effectively prevent stem rust provided the applications were made at the proper time and with the right kind of dust. Not only was the rust prevented, but grain yields were considerably increased. Very little is known, however, about the number of applications that would be necessary, the time of application, and the effect of dusting in different

[1] The experiments were made cooperatively between the Niagara Sprayer Company and the University of Minnesota, under a special grant made by the Niagara Sprayer Company to the University.

[2] Published with the approval of the Director as Paper No. 962 of the Journal Series of the Minnesota Agricultural Experiment Station.

[3] The writer here expresses his appreciation to Dr. E. C. Stakman, Professor of Plant Pathology, University of Minnesota, and Pathologist, Bureau of Plant Industry, United States Department of Agriculture, for his helpful advice and criticism; to Dr. E. B. Lambert, Associate Pathologist, Bureau of Plant Industry, United States Department of Agriculture, for his helpful advice in outlining the project; to Mr. C. V. Kightlinger, formerly Agent, Bureau of Plant Industry, United States Department of Agriculture, for his assistance in taking notes at St. Paul, Morris, and Crookston, Minnesota; to Mr. R. S. Dunham, Agronomist at the N. W. School and Station, Crookston, Minnesota; and to Mr. R. O. Bridgford, Agronomist at the West Central School and Station, Morris, Minnesota, for their cooperation and assistance at their respective stations.

seasons. The writer's experiments were made in order to obtain additional and more definite information on some of these questions.

Specifically, the objects of the writer's experiments were to determine, in a preliminary way, the most effective and practicable number, time, rate, and method of application of Kolo sulphur dust for the control of stem and leaf rusts and the subsequent effect on yield, bushel weight, grade, protein content, and the setting of seed of wheat. Experiments were conducted in 1927 at Morris, Crookston, and St. Paul, Minnesota.

MATERIALS AND METHODS

Varieties of wheat most extensively grown in Minnesota were used in these tests. Marquis and Ruby, common bread wheats, and Mindum, a durum wheat, were sown with an 8-foot nursery seeder at a uniform rate of approximately 90 pounds an acre. When the young plants were about 12 inches high, the seeded areas were divided into plots 8 feet square, separated from one another by 1-foot alleys. More than 2,000 plots were included in the experiment.

Kolo dust, a colloidal sulphur dust, supplied by the Niagara Sprayer Company of Middleport, N. Y., was used throughout the experiments. The dust was applied by means of a hand duster (Niagara Blower Gun No. 42, at Morris and St. Paul, and a Root Hand Gun, at Crookston). The hand dusters were calibrated so that 10, 20, 30, and 40 revolutions of the crank, made at the rate of one revolution a step or pace in walking around the plot, discharged dust at the rates of 15, 30, 45, or 60 pounds per acre per application, respectively. By the expressions 15-, 30-, 45-, or 60-pound rate, used hereafter in this paper, is meant the amount of dust used per acre per application. The dusters were checked at frequent intervals throughout the experiments to insure reasonable accuracy and uniformity in the rates of application.

The experiments were made on the following soil types: Hempsted silt loam at St. Paul, Clarion silt loam at Morris, and Fargo clay loam at Crookston. The soil in the plots at each of these places appeared to be fairly homogeneous.

Stem-rust data were obtained by making estimates of the average amount of rust on the plants in each plot according to the scale adopted by the Office of Cereal Crops and Diseases, United States Department of Agriculture. Separate notes were taken on the amount of rust on the "neck" or peduncle, sheath or boot, and the lowest internode.

As there appeared to be no significant and consistent differences in the amount of rust on the sheaths or boots and on the lowest internodes of plants in the various plots, only the data on severity of rust infection on the peduncle will be given. It is the peduncle of the plant that we are chiefly interested in protecting for practical purposes, since stem rust causes

greatest damage on this part of the plant. Rust usually is already present on the sheath or boot and on the lowest internode, when practical dusting operations ordinarily would be started, and dusting can not be expected to control rust on those parts of the plant already infected when dusting is begun. Therefore, the importance of protecting the peduncle seems paramount.

When the wheat was ripe, square-yard samples were harvested from each plot and later threshed with a nursery thresher. The seed from each plot was weighed and the yield in bushels per acre was then computed. The difference in yield might have been greater if an ordinary threshing machine had been used. Undoubtedly more of the light, shrunken seed from the nondusted plots would have been blown into the straw stack had the grain been threshed with a field machine.

Determinations of the weight per bushel of wheat from each plot were made by the methods recommended by the United States Grain Standards Act, except that, since the samples were small, a one-half-pint tester was used instead of the regular one-quart tester.

The samples were graded according to the official grain standards recommended by the United States Grain Standards Act.

From the data on the yield of wheat from the nondusted plots at the various localities, a probable error for each experiment was calculated by the use of the following formula: $P.E. = \pm .6745 \sqrt{\frac{Ef.d^2}{n}}$ where d = deviation in bushels of a class from the mean, f = frequency, n = total number of individuals, and N = the number of replications. The following probable errors were obtained for Marquis wheat:

Locality	Variety	P. E. in per cent
Morris	Marquis	5.3087
St. Paul	" (early planting)	5.8386
"	" (late planting)	9.9930
Crookston	"	7.1233

The average probable error for Morris, Crookston, and St. Paul =

$$1/3\sqrt{5.3^2 + 5.8^2 + 7.1^2} = 3.53\%.$$

The average probable error for Crookston and St. Paul (whenever Morris data were not obtained) $= 1/2\sqrt{5.3^2 + 7.1^2} = 4.58\%$.

Random samples of seed from the plots dusted for the control of leaf rust were analyzed for protein content by the Kjeldahl method for nitrogen analysis.

For the schedule consisting of three applications of Kolo dust at the four different rates there are six different times of application, as follows:

First Application	*Second Application*	*Third Application*
1. Flowering time	4 days after first	4 days after second
2. " "	6 " " "	" " " "
3. " "	8 " " "	" " " "
4. 5 days after flowering time	4 " " "	" " " "
5. " " " " "	6 " " "	" " " "
6. " " " " "	8 " " "	" " " "

For the schedule consisting of two applications of Kolo dust at the four different rates, there are eight different times of application, as follows:

First Application	*Second Application*
1. Flowering time	4 days after first
2. " "	6 " " "
3. " "	8 " " "
4. " "	10 " " "
5. 5 days after flowering time	4 " " "
6. " " " " "	6 " " "
7. " " " " "	8 " " "
8. " " " " "	10 " " "

For the schedule consisting of one application of Kolo dust at the four different rates, there are four different times of application, as follows:

Treatments	*Time of Application*
First	At flowering time
Second	5 days after flowering time
Third	10 " " " "
Fourth	15 " " " "

The fact that the stem-rust epiphytotic was very severe at Morris in 1927 afforded a good opportunity to test the effectiveness of dusting with Kolo dust. The rust developed rapidly, especially during the latter part of the growing season, and, together with hot, dry weather, decreased yield and quality of wheat in this district very materially. At Crookston, about 200 miles north of Morris, the epiphytotic was not so destructive. The development of rust was checked to a considerable extent by cool weather during the first two weeks in August. At St. Paul, about 150 miles east and slightly south of Morris, the epiphytotic was almost as severe as at Morris, although grain probably was damaged less by hot dry weather.

Marquis wheat was sown at Morris on April 25, at Crookston on April 30, and at St. Paul on May 2. The wheat plants flowered rather uniformly;

at Morris on July 6, at Crookston July 11, and at St. Paul on July 8. Flowering-time applications were made on these dates. Rust notes were taken at Morris on August 1, at Crookston on August 12, and at St. Paul on August 2. The plots were harvested at Morris on August 9, at Crookston on August 19 to 23, and at St. Paul on August 14.

THE EFFECT OF THREE APPLICATIONS OF KOLO DUST ON MARQUIS WHEAT IN 1927

The mean average percentages of stem rust on the peduncle of the wheat plant were significantly lower on the dusted than on the nondusted plots at the three stations (Table 1). Dusting controlled rust so well at Morris that the dusted plots easily could be singled out from a distance of 100 feet. The bright and clean appearance of the plants in the dusted plots contrasted sharply with the dull, dark, dead appearance of those in the nondusted plots. The mean average percentage of rust in the nondusted plots ranged from 60.9 to 68.3 in comparison with 13.0 to 41.3 in the dusted plots. As a rule, the mean average percentage was lower in those plots dusted first at flowering time than in those in which the first application was made 5 days later. It should be pointed out that the results of making the first application at flowering time were better in Crookston than at Morris and St. Paul, although the exact reason is not known.

The best schedule at the three stations, from the standpoint of time of application, was the F.T. + 6 or 8 + 4, which means that the first application was made at flowering time, the second either 6 or 8 days after the first, and the third 4 days after the second. For the F.T. + 6 + 4 schedule the mean average percentages of rust on the plots dusted at the 15-, 30-, 45-, and 60-pound rates were 26.1, 21.9, 18.8, and 14.0 per cent, respectively (Table 1, Plots 56, 63, 70, and 77). For the F.T. + 8 + 4 schedule at the 15-, 30-, 45-, and 60-pound rates, the mean average percentages were 23.6, 17.6, 15.4, and 13.0 per cent, respectively (Table 1, Plots 57, 64, 71, and 78). From this it is evident that the 60-pound rate was considerably more effective than the 15-pound rate but probably not significantly more effective than the 30- and 45-pound rates. It also seems that there was no significant difference in the mean average percentage of rust in plots dusted according to the F.T. + 6 + 4 and F.T. + 8 + 4 schedules.

The mean average yields of wheat were uniformly and significantly higher on the dusted than on the nondusted plots at the three stations. For the near-by nondusted plots the mean average yields were 24.57 $\pm$ 0.87, 25.16 $\pm$ 0.89, 24.33 $\pm$ 0.86, 21.48 $\pm$ 0.76, and 23.26 $\pm$ 0.82 bushels per acre, respectively (Table 1, Plots 54, 61, 68, 75, and 82). The average yield for all the nondusted plots at the three stations, 120 in all, was 23.31 bushels per acre. The plots on which the first application was made at flowering

TABLE 1.—*The effect of 3 applications of Kolo dust applied at 15–, 30–, 45–, and 60-pound rates and at various intervals in relation to flowering time of wheat plant on the control of stem rust, increase in yield, weight per bushel, and grade of Marquis wheat at Morris, Crookston, and St. Paul, Minnesota, in 1927*

Plot No.	Application		Average[b] percentage of stem rust on peduncle				Average[b] yield in bushels per acre				Average[b] weight in pounds per bushel				Average[b] grade number			
	Rate in pounds per acre	Time[a]	M	C	St. P	Mean av.	M	C	St. P	Mean average	M	C	St. P	Mean av.	M	C	St. P	Mean av.
54	0	Check	77.5	72.5	55.0	68.3	19.91	28.54	25.25	24.57 ± 0.87	48.5	53.8	51.3	51.2	S. G.[c]	4	5	5
55	15	FT + 4 + 4	25.0	67.5	28.8	40.4	27.47	30.67	28.63	28.92 ± 1.02	54.9	55.4	53.8	54.7	3	3	4	3
56	15	" + 6 + 4	20.8	35.0	22.5	26.1	27.43	36.67	30.76	31.62 ± 1.12	55.4	56.0	54.5	55.3	3	3	3	3
57	15	" + 8 + 4	10.8	35.0	25.0	23.6	28.05	35.65	30.72	31.47 ± 1.11	56.9	56.5	54.5	56.0	2	2	3	3
58	15	(FT + 5) + 4 + 4	31.3	18.8	36.3	28.8	26.14	40.14	28.96	31.75 ± 1.12	54.0	58.9	53.3	55.4	4	1	4	3
59	15	" + 6 + 4	36.3	25.0	41.3	34.2	24.41	39.16	27.07	30.21 ± 1.07	54.4	56.8	52.5	54.6	4	2	4	3
60	15	" + 8 + 4	43.8	46.3	38.8	41.3	23.47	42.23	26.40	30.70 ± 1.08	53.5	56.0	53.0	54.2	4	3	4	4
61	0	Check	81.3	68.8	47.5	65.9	20.45	29.96	25.07	25.16 ± 0.89	49.0	55.8	49.3	51.4	S. G.	3	S. G.	5
62	30	FT + 4 + 4	23.8	45.0	30.0	32.9	24.49	35.03	31.52	30.35 ± 1.07	55.5	57.4	52.5	55.1	3	2	4	3
63	30	" + 6 + 4	17.0	22.5	26.3	21.9	25.87	40.45	32.81	33.04 ± 1.17	55.5	57.7	55.0	56.1	3	1	3	3
64	30	" + 8 + 4	11.5	20.0	21.3	17.6	30.72	40.41	31.92	34.35 ± 1.21	56.4	58.0	54.3	56.2	3	1	4	3
65	30	(FT + 5) + 4 + 4	33.8	7.0	21.3	20.7	24.23	38.20	29.16	30.53 ± 1.08	54.6	59.0	52.5	55.4	3	1	4	3
66	30	" + 6 + 4	36.3	14.3	35.0	28.5	22.98	35.92	25.56	28.15 ± 0.99	53.3	57.8	53.5	54.9	4	1	4	3
67	30	" + 8 + 4	50.0	41.3	18.8	36.7	21.78	31.92	29.72	27.81 ± 0.98	51.9	56.6	54.0	54.2	5	2	4	4
68	0	Check	81.3	82.5	38.8	67.9	21.02	32.45	19.52	24.33 ± 0.86	47.4	54.3	48.3	50.0	S. G.	4	S. G.	5
69	45	FT + 4 + 4	27.5	55.0	21.3	34.6	27.83	42.23	26.63	32.23 ± 1.14	54.8	57.5	52.5	54.9	3	1	4	3
70	45	" + 6 + 4	21.3	18.8	16.3	18.8	27.92	43.52	29.61	33.68 ± 1.19	56.0	58.4	54.3	56.2	3	1	4	3
71	45	" + 8 + 4	13.8	17.5	15.0	15.4	30.36	36.36	24.63	30.45 ± 1.08	57.1	57.5	52.8	55.8	2	1	4	3
72	45	(FT + 5) + 4 + 4	30.0	10.0	18.8	19.6	26.18	32.45	25.87	28.17 ± 0.99	54.3	56.9	52.3	54.5	4	2	5	3
73	45	" + 6 + 4	31.3	17.5	22.5	23.8	27.78	29.87	25.74	27.80 ± 0.98	54.3	57.4	53.0	54.9	4	2	4	3
74	45	" + 8 + 4	45.0	43.8	20.0	36.3	24.32	36.03	27.78	29.38 ± 1.04	53.2	56.6	54.5	54.8	4	2	3	3
75	0	Check	82.5	63.8	36.3	60.9	17.47	25.29	22.67	21.48 ± 0.76	47.2	51.8	51.0	50.0	S. G.	5	5	5
76	60	FT + 4 + 4	27.5	48.8	21.3	32.5	30.14	31.61	33.61	31.79 ± 1.12	55.9	56.0	55.8	55.9	3	3	3	3
77	60	" + 6 + 4	22.5	10.8	8.8	14.0	32.18	44.54	32.09	36.27 ± 1.28	55.0	57.6	58.8	57.1	3	1	1	2
78	60	" + 8 + 4	13.8	13.8	11.3	13.0	33.47	38.41	30.67	34.18 ± 1.21	57.1	56.3	58.0	57.1	2	3	1	2
79	60	(FT + 5) + 4 + 4	35.0	11.3	17.5	21.3	27.74	39.39	31.21	32.78 ± 1.16	53.0	56.0	55.0	54.7	4	3	3	3
80	60	" + 6 + 4	30.0	15.8	22.5	22.8	26.19	36.67	28.67	30.51 ± 1.08	54.9	53.9	52.5	53.8	3	4	4	4
81	60	" + 8 + 4	43.8	28.8	18.8	30.5	24.67	34.94	29.79	29.80 ± 1.05	51.1	55.3	54.8	53.7	5	3	3	4
82	0	Check	85.0	61.3	36.3	60.9	18.27	28.85	22.67	23.26 ± 0.82	48.1	54.9	51.0	51.3	S. G.	3	5	5

[a] FT + 4 + 4 = First application at flowering time, the second 4 days after the first, and the third 4 days after the second.
" + 6 + 4 = " " " " " " " 6 " " " " " " " 4 " " " "
" + 8 + 4 = " " " " " " " 8 " " " " " " " 4 " " " "
(FT + 5) + 4 + 4 = " " 5 days after " " " " 8 " " " " " " " 4 " " " "
(FT + 5) + 6 + 4 = " " 5 days after " " " " 8 " " " " " " " 4 " " " "
(FT + 5) + 8 + 4 = " " 5 days after " " " " 8 " " " " " " " 4 " " " "

[b] Average of four systematically distributed plots at each locality.

[c] S. G. = Sample Grade.

time, in addition to being freer from stem rust, yielded consistently more wheat than those plots on which the first application was made 5 days after flowering time. For the F.T. + 6 + 4 schedule the mean average yields of wheat from these plots dusted at the 15-, 30-, 45-, and 60-pound rates were 31.62 ± 1.12, 33.04 ± 1.17, 33.68 ± 1.19, and 36.27 ± 1.28 bushels per acre, respectively (Table 1, Plots 56, 63, 70, and 77). These yields, when compared with the average yield of 23.31 bushels per acre from the 120 nondusted plots, represent an increase of 8.31, 9.73, 10.37, and 12.96 bushels per acre, or increases of 35.6, 41.7, 44.5, and 55.6 per cent, respectively. For the F.T. + 8 + 4 schedule the mean average yields for the plots dusted at the 15-, 30-, 45-, and 60-pound rates were 31.47 ± 1.11, 34.35 ± 1.21, 30.45 ± 1.08, and 34.18 ± 1.21 bushels per acre, respectively (Table 1, Plots 57, 64, 71, and 78). These yields, when compared with those of the nondusted plots, represent an increase of 8.16, 11.04, 7.14, and 10.87 bushels per acre, or increases of 35.0, 47.4, 30.6, and 46.6 per cent, respectively. The highest yielding plots, which were dusted three times at Morris, Crookston, and St. Paul, when considered separately, yielded 76, 52, and 44 per cent more than the near-by nondusted plots, respectively. From these data it is seen that the 15- and 30-pound rates were almost as effective as the 45- and 60-pound rates. This again emphasizes the fact that timeliness of application is one of the most important factors to be considered. At Crookston, when the first application was made 5 days after flowering time, the yield was almost as high as when the first application was made at flowering time, but this was not true at Morris and St. Paul.

The mean average weights per bushel and grades of wheat were uniformly and significantly higher for the dusted than for the nondusted plots at the three stations. For the near-by nondusted plots the mean average weights were 51.2, 51.4, 50.0, 50.0, and 51.3 pounds, respectively (Table 1, Plots 54, 61, 68, 75, and 82), and the mean average grade for the wheat from each was No. 5. The average weight per bushel for the 120 nondusted plots at the three stations was 50.2 pounds, grading No. 5. For the F.T. + 6 + 4 schedule the mean average weights for the wheat from the plots dusted at the 15-, 30-, 45-, and 60-pound rates were 55.3, 56.1, 56.2, and 57.1 pounds, and on the average graded No. 3 for the first three rates and No. 2 for the last (Table 1, Plots 56, 63, 70, and 77). These weights, when compared with the average weight of 50.2 pounds for the 120 nondusted plots, represent increases in weight of 5.1, 5.9, 6.0, and 6.9 pounds per bushel, respectively, as well as an increase in grade from No. 5 to Nos. 3 and 2. For the F.T. + 8 + 4 schedule the mean average weights of wheat from the plots dusted at the 15-, 30-, 45-, and 60-pound rates were 56.0, 56.2, 55.8, and 57.1 pounds per bushel, grading the same as in the F.T. + 8 + 4 schedule (Table 1, Plots 57, 64, 71, and 78). These weights, when compared with 50.2 pounds, the average of 120 nondusted plots at the three

stations, represent increases in weight of 5.8, 6.0, 5.6, and 6.9 pounds per bushel, respectively, as well as an increase in grade from No. 5 to Nos. 3 and 2. As a rule, the weight per bushel was higher for the plots receiving the first application at flowering time than for those receiving it 5 days later. From these data it is evident that the 15-, 30-, and 45-pound rates were about equally effective, whereas the wheat from the plots dusted at the 60-pound rate weighed and graded uniformly slightly higher than that from those dusted at the lower rates. It is doubtful, however, whether the increase in weight per bushel and grade of wheat from plots receiving 3 applications at the 60-pound rate, 180 pounds in all, would pay for the additional dust and labor, especially since 3 applications at the 15-pound rate, 45 pounds in all, prove almost as effective.

THE EFFECT OF TWO APPLICATIONS OF KOLO DUST ON MARQUIS WHEAT

The mean average percentages of stem rust on the peduncle of the wheat plants were markedly lower on the dusted than on the nondusted plots at the three stations. The mean average percentages on the near-by nondusted plots ranged from 64.5 to 63.3, whereas on the dusted plots they ranged from 28.6 to 54.2. The differences in the mean average percentages of rust on the plots where the first application was made either at flowering time or 5 days later were not very great, although there appeared to be slightly less rust in the former case, especially at Morris and St. Paul. From the examination of table 2, it is apparent that there are no significant differences in the mean average percentages of rust on the plots dusted at the 15-, 30-, 45-, and 60-pound rates.

Since the amount of rust at Morris was the lowest in the plots dusted first at flowering time, followed by a second dusting 6 or 8 days later, only these plots were harvested along with all the plots dusted at the 60-pound rate. At Crookston and St. Paul all the plots dusted twice were harvested. The mean average yields were consistently higher on the dusted than on the nondusted plots. The mean average yields on the near-by nondusted plots compare favorably with the average of all the nondusted plots in the entire experiment. For the F.T. + 6 schedule the mean average yields for the plots dusted at the 15-, 30-, 45-, and 60-pound rates were, respectively, higher than those of the nondusted plots. For the F.T. + 8 schedule where the second application was made 8 instead of 6 days after the flowering-time application, the mean average yields for the plots dusted at the above rates showed increase of 30.0, 23.3, 39.7, and 29.6 per cent, respectively, in comparison with that of the nondusted plots. A careful examination of these data and the data given for the F.T. + 6 schedule shows that the 15- and 30-pound rates were practically as effective as the 45- and 60-pound rates, as measured by increases in yield. Further comparisons of the relative efficiency of the two applications when applied at the former rates are

TABLE 2.—*The effect of 2 applications of Kolo dust applied at 15-, 30-, 45-, and 60-pound rates and at various intervals in relation to flowering time of wheat plant on the control of stem rust, increase in yield, weight per bushel, and grade of Marquis wheat at Morris, Crookston, and St. Paul, Minnesota, in 1927*

Plot No.	Application		Average[b] percentage of stem rust on peduncle				Average[b] yield in bushels per acre				Average[b] weight in pounds per bushel				Average[b] grade number			
	Rate in pounds per acre	Time[a]	M.	C.	St. P	Mean av.	M	C	St. P	Mean average	M	C	St. P	Mean av.	M	C	St. P	Mean av.
18	0	Check	75.0	77.5	46.3	66.3	18.49	25.51	20.00	21.33 ± 0.75	45.9	54.8	48.5	49.7	S. G.[c]	3	S. G.	5
19	15	FT + 4	40.0	68.8	23.8	44.2	.....	27.11	24.60	25.86 ± 1.18	.....	56.0	50.3	53.2	.....	3	5	4
20	15	" + 6	32.5	62.5	18.8	37.9	28.58	35.07	26.63	30.09 ± 1.06	55.9	56.8	53.0	55.2	3	2	4	3
21	15	" + 8	28.8	68.8	22.5	40.0	25.29	36.41	29.24	30.31 ± 1.07	55.6	57.0	53.8	55.5	3	2	4	3
22	15	" + 10	42.5	55.0	25.0	40.8	.....	37.96	29.57	33.77 ± 1.55	.....	57.1	54.3	55.7	.....	2	4	3
23	30	" + 4	40.0	66.3	21.3	32.3	.....	36.23	30.36	22.20 ± 1.02	.....	55.9	55.3	55.6	.....	3	3	3
24	30	" + 6	31.3	67.5	16.3	38.4	29.14	31.03	34.05	31.41 ± 1.11	54.1	56.3	56.5	55.6	4	3	2	3
25	30	" + 8	32.5	56.3	26.3	38.4	28.19	27.74	30.27	28.73 ± 1.01	55.4	55.0	56.3	55.6	3	3	3	3
26	30	" + 10	41.3	17.0	27.5	28.6	.....	36.98	30.49	33.74 ± 1.55	.....	57.8	55.0	56.4	.....	1	3	3
27	0	Check	72.5	77.5	45.0	65.0	18.54	25.83	21.69	22.02 ± 0.78	47.1	54.1	49.3	50.2	S. G.	4	S. G.	5
28	45	FT + 4	42.5	76.3	43.8	54.2	.....	33.65	26.58	30.11 ± 1.38	.....	55.6	53.5	54.6	.....	3	4	3
29	45	" + 6	42.5	72.5	41.3	52.1	29.43	33.29	31.87	31.53 ± 1.11	55.3	56.3	55.3	55.6	3	3	3	3
30	45	" + 8	32.5	58.8	23.8	38.4	28.54	35.52	33.65	32.57 ± 1.15	56.3	55.1	53.5	55.0	3	3	4	3
31	45	" + 10	47.5	15.0	23.8	28.8	.....	38.01	32.50	35.26 ± 1.62	.....	56.6	54.8	55.7	.....	2	3	3
32	60	" + 4	43.8	50.0	38.8	44.2	24.41	28.72	29.47	27.53 ± 0.97	55.4	55.1	52.8	54.4	3	3	4	4
33	60	" + 6	30.0	56.3	28.8	38.4	25.70	31.52	31.60	29.61 ± 1.05	55.8	54.5	52.5	54.3	3	3	4	4
34	60	" + 8	31.3	41.3	26.3	32.6	23.61	33.87	33.14	30.21 ± 1.07	56.3	55.5	53.3	55.0	3	3	4	3
35	60	" + 10	45.0	25.0	35.0	35.0	20.67	38.45	30.38	29.50 ± 1.04	55.7	56.6	55.0	55.8	3	2	3	3
36	0	Check	72.5	72.5	48.8	64.6	16.86	28.00	25.60	23.49 ± 0.83	44.9	53.0	48.5	48.5	S. G.	4	S. G.	S. G.
37	15	(FT + 5) + 4	51.3	30.0	31.3	34.2	.....	34.14	25.16	29.65 ± 1.36	.....	55.6	51.5	53.6	.....	3	5	4
38	15	" + 6	51.3	43.8	40.0	45.0	.....	29.92	22.94	26.43 ± 1.21	.....	54.4	49.5	52.0	.....	4	5	5
39	15	" + 8	52.5	45.0	27.5	41.7	.....	26.45	26.00	26.23 ± 1.20	.....	53.5	51.3	52.4	.....	4	5	5
40	15	" + 10	53.8	65.0	33.8	50.9	.....	31.36	20.40	25.88 ± 1.19	.....	53.0	45.3	49.2	.....	4	S. G.	S. G.
41	30	" + 4	60.0	21.3	28.8	36.7	.....	43.79	22.71	33.25 ± 1.52	.....	56.0	49.8	52.9	.....	3	5	4
42	30	" + 6	60.0	35.0	28.8	41.3	.....	39.52	22.66	31.09 ± 1.42	.....	55.4	51.8	53.6	.....	3	5	4
43	30	" + 8	57.5	53.8	21.3	44.2	.....	35.25	23.78	29.52 ± 1.35	.....	55.3	51.0	53.2	.....	4	5	4
44	30	" + 10	62.5	62.5	26.3	50.4	.....	33.63	19.74	26.69 ± 1.22	.....	54.4	46.7	50.6	.....	4	S. G.	5
45	0	Check	77.5	77.5	38.8	64.6	18.98	27.07	22.14	22.73 ± 0.80	47.8	52.7	48.5	49.7	.....	4	S. G.	4
46	45	(FT + 5) + 4	55.0	13.8	25.0	31.3	.....	35.88	23.56	29.72 ± 1.36	.....	57.1	50.8	54.0	.....	2	5	4

TABLE 2.—*Continued*

Plot No.	Application		Average[b] percentage of stem rust on peduncle				Average[b] yield in bushels per acre				Average[b] weight in pounds per bushel				Average[b] grade number			
	Rate in pounds per acre	Time[a]	M	C	St. P	Mean av.	M	C	St. P	Mean average	M	C	St. P	Mean av.	M	C	St. P	Mean av.
47	45	(FT + 5) + 6	55.0	30.0	23.8	36.3	.....	38.23	24.54	31.39 ± 1.44	.....	56.5	50.3	53.4	.....	2	5	4
48	45	" + 8	50.0	51.3	15.0	38.8	.....	36.27	27.30	31.78 ± 1.46	.....	56.5	51.5	54.0	.....	2	5	4
49	45	" + 10	52.5	67.5	22.5	47.5	.....	29.38	24.40	26.89 ± 1.23	.....	54.3	52.3	53.3	.....	4	5	4
50	60	" + 4	52.5	18.8	18.8	30.0	22.32	35.47	30.41	29.40 ± 1.04	50.2	57.0	54.54	53.9	5	2	3	4
51	60	" + 6	51.3	28.8	21.3	33.8	23.21	39.16	25.60	29.32 ± 1.03	52.6	57.4	54.8	54.9	4	2	3	3
52	60	" + 8	47.5	46.3	23.8	39.2	23.16	35.78	31.38	30.11 ± 1.06	51.1	57.3	55.3	54.6	5	2	3	3
53	60	" + 10	50.0	63.8	35.0	49.6	23.82	28.72	29.92	27.49 ± 0.97	51.8	56.5	54.0	54.1	5	2	4	4
54	0	Check	77.5	72.5	55.0	68.3	19.91	28.54	25.25	24.57 ± 0.87	48.5	53.8	51.3	51.2	S. G.	4	5	5

[a] FT + 4 = First application at flowering time and the second 4 days after the first application.
" + 6 = " " " " " " " 6 " " " " "
" + 8 = " " " " " " " 8 " " " " "
" + 10 = " " " " " " " 10 " " " " "
(FT + 5) + 4 = " " 5 days after " " " " " 4 " " " " "
" + 5) + 6 = " " " " " " " " 6 " " " " "
" + 5) + 8 = " " " " " " " " 8 " " " " "
" + 5) + 10 = " " " " " " " " 10 " " " " "

[b] Average of four systematically distributed plots at each locality.
[c] S. G. = Sample Grade.

not justifiable, since the mean average yield data for the plots dusted on the other schedules do not include the Morris data, where the yields on all the plots were much lower than at either Crookston or St. Paul. This then would necessarily give higher mean average yields. The highest-yielding plots receiving two applications of Kolo dust at Morris, Crookston, and St. Paul, when considered separately, yielded 58, 38, and 51 per cent more than the near-by nondusted plots, respectively. These increases of 58, 38, and 51 per cent for the highest-yielding plots dusted twice at Morris, Crookston, and St. Paul compare favorably with the increases of 76, 52, and 44 per cent for the highest-yielding plots dusted three times at the same stations, although, with the exception of those at St. Paul, they are not so high (Table 4).

The mean average weights per bushel and the grades were higher for the dusted than for the nondusted plots at the three stations. The mean average weights for the near-by nondusted plots were 49.7, 50.2, 48.5, 49.7, and 51.2 pounds, respectively (Table 2, Plots 18, 27, 36, 45, and 54). The mean average grade of each near-by nondusted plot was No. 5. Wheat in the F.T. + 6 schedule averaged grade No. 3 for the 15-, 30-, and 45-pound rates and No. 4 for the 60-pound rate, while its mean average represented increases of 5.0, 5.4, 5.4, and 4.1 pound per bushel, respectively. For the F.T. + 8 schedule where the second application was made 8 instead of 6 days after the flowering-time application, the mean average weights represented an increase of 5.3, 5.4, 4.8, and 4.8 pounds, respectively, as well as an increase in grade from No. 5 up to a No. 3 in comparison with that of the nondusted plots. Further comparisons of the efficiency of two applications when applied on the other schedules are not justifiable since the mean average weight per bushel and grade do not include the Morris data, where the weight per bushel and grade were much lower than at Crookston and St. Paul. From the data presented it is evident, however, that two applications at the 15-, 30-, 45-, and 60-pound rate were about equally effective. It should be pointed out here that the increases in mean average weight per bushel and grade of wheat from plots receiving two applications of Kolo dust compare very favorably with those from the plots receiving three applications (Table 4).

THE EFFECT OF ONE APPLICATION OF KOLO DUST ON MARQUIS WHEAT

The mean average percentages of stem rust on the peduncle of the wheat plant (Table 3) were slightly lower on the plots receiving one application of Kolo dust than on the nondusted plots. However, the differences do not seem significant and they do not even constitute practical control. The mean average percentages of rust on the near-by nondusted plots were 71.3 and 66.3, respectively (Table 3, Plots 9 and 18). The mean average percentages on the plots dusted once ranged from 40.8 to 62.1. The differ-

TABLE 3.—*The effect of 1 application of Kolo dust applied at 15-, 30-, 45-, and 60-pound rates and at various intervals in relation to flowering time of wheat plants on the control of stem rust, increase in yield, weight per bushel, and grade of Marquis wheat at Morris, Crookston, and St. Paul, Minnesota, in 1927*

Plot No.	Application		Average[b] percentage of stem rust on peduncle				Average[b] yield in bushels per acre				Average[b] weight in pounds per bushel				Average[b] grade number			
	Rate in pounds per acre	Time[a]	M	C	St. P	Mean av.	M	C	St. P	Mean average	M	C	St. P	Mean av.	M	C	St. P	Mean av.
1	15	FT	51.3	78.8	37.5	55.9	.....	35.96	27.73	31.85 ± 1.46	.....	56.5	51.3	53.9	.....	2	5	4
2	15	(FT + 5)	55.0	75.0	36.3	55.4	.....	34.45	25.91	30.18 ± 1.38	.....	56.1	48.5	52.3	.....	3	S. G.	5
3	15	(FT + 10)	57.5	40.0	41.3	46.3	.....	35.30	24.81	30.06 ± 1.36	.....	56.8	51.8	54.3	.....	2	5	4
4	15	(FT + 15)	60.0	77.5	48.8	62.1	.....	29.38	26.45	27.92 ± 1.28	.....	54.9	52.0	53.5	.....	3	5	4
5	30	FT	52.5	75.0	37.5	55.0	.....	32.72	26.36	29.54 ± 1.35	.....	56.0	51.8	53.9	.....	3	5	4
6	30	(FT + 15)	57.5	70.0	42.5	56.7	.....	28.40	27.07	27.74 ± 1.27	.....	55.1	52.8	54.0	.....	3	4	4
7	30	(FT + 10)	50.0	43.8	37.5	43.8	.....	33.65	24.67	29.16 ± 1.34	.....	57.3	52.8	55.0	.....	2	4	3
8	30	(FT + 15)	50.0	78.8	41.3	56.7	.....	27.65	25.52	26.59 ± 1.22	.....	53.3	50.3	51.8	.....	4	5	5
9	0	Check	76.3	85.0	52.5	71.3	18.36	31.20	24.63	24.73 ± 0.87	46.8	53.5	50.0	50.1	S. G.[c]	4	5	5
10	45	FT	52.5	85.0	33.8	57.1	.....	32.45	26.52	29.49 ± 1.35	.....	55.1	52.8	54.0	.....	3	4	4
11	45	(FT + 5)	47.5	80.0	43.8	57.1	.....	32.85	23.96	28.41 ± 1.30	.....	55.4	49.5	52.5	.....	3	5	4
12	45	(FT + 10)	60.0	57.5	30.0	49.2	.....	33.52	22.45	27.99 ± 1.28	.....	55.9	51.0	53.5	.....	3	5	4
13	45	(FT + 15)	57.5	68.8	40.0	55.4	.....	30.98	21.38	26.18 ± 1.20	.....	54.5	50.0	52.5	.....	3	5	4
14	60	FT	50.0	76.3	25.0	50.4	26.98	33.34	26.85	29.06 ± 1.03	53.7	55.6	51.3	53.5	4	3	5	4
15	60	(FT + 5)	51.3	76.3	30.0	52.5	29.47	38.10	21.03	29.53 ± 1.04	52.6	57.1	49.5	53.1	4	2	5	4
16	60	(FT + 10)	52.5	42.5	27.5	40.8	26.67	28.14	22.18	25.66 ± 0.91	51.1	57.5	50.3	52.6	5	1	5	4
17	60	(FT + 15)	50.0	70.0	33.8	51.3	24.40	30.22	24.05	26.22 ± 0.93	54.3	56.4	49.8	53.5	4	3	5	4
18	0	Check	75.0	77.5	46.3	66.3	18.49	25.51	20.00	21.33 ± 0.75	45.9	54.8	48.5	49.7	S. G.	3	S. G.	5

[a] FT = One application at flowering time.
(FT + 5) = One application 5 days after flowering time.
(FT + 10) = " " 10 " " " "
(FT + 15) = " " 15 " " " "

[b] Average of four systematically distributed plots at each locality.

[c] S. G. = Sample Grade.

ences in the mean average percentage of rust on the plots dusted once on the different schedules and at the different rates were not large enough to permit any definite conclusions except that the rust percentage was uniformly the lowest on the plots where the Kolo dust was applied 10 days after flowering time. This, as already pointed out, may have been due to the fact that at Crookston all the plots dusted 8 to 10 days after flowering time were freer from rust than the other plots; consequently, this would lower the mean average percentage of rust for these particular plots.

The mean average yields of wheat from the near-by nondusted plots were 24.72 ± 0.87 and 21.33 ± 0.75 bushels per acre, respectively (Table 3, Plots 9 and 18). These yields compare favorably with 23.31 bushels per acre for the average yield of all the nondusted plots in the entire experiment. We will compare only the yield data for the plots dusted once at the 60-pound rate, since only these particular plots were harvested at Morris. In general, at the three stations, plots dusted once, especially at flowering time, outyielded the nondusted plots. The result would hardly be expected from one application of Kolo dust, since there appeared to be no significant observable evidences of the practical control of rust at the time the rust notes were taken shortly before harvest. This may be a coincidence, but more probably it may have been due to the fact that one application of Kolo dust delayed the development of the rust for a time, although the final percentage was not significantly reduced in comparison with that in the nondusted plots. The mean average yields for the plots dusted once at the 60-pound rate at the flowering time, 5, 10, and 15 days after flowering time showed increases of 5.75, 6.22, 2.35, and 2.91 bushels per acre, respectively, of which only the yields for the plots dusted once at flowering time or 5 days after flowering time are probably significant. It would not be justifiable to draw any conclusions regarding the efficiency of one application at the 15-, 30-, 45-, and 60-pound rates because only the plots dusted at the 60-pound rate were harvested at Morris. However, if the data at Crookston and St. Paul are considered separately or together, there appear to be no significant differences in yield for the four rates of application. The time of application is a far more important factor.

The mean average weights per bushel and the grade of wheat at the three stations were uniformly higher for the plots dusted once than for the nondusted plots. The mean average weights per bushel for the near-by nondusted plots were 50.1 and 49.7 pounds, respectively (Table 3, Plots 9 and 18), and the mean average grade of each was No. 5. These compare favorably with 50.2 pounds per bushel and a grade of No. 5 for the average of 120 nondusted plots in the entire experiment. As in discussing the mean average yields, only the plots dusted once at the 60-pound rate will be considered in a comparison of the mean average weight per bushel and the grade. In each case the mean average grade was No. 4. The weights when

TABLE 4.—*Comparison of mean-average data upon the efficiency of 3, 2, and 1 applications of Kolo dust when applied at rates of 15-, 30-, 45-, and 60-pounds per acre and at various intervals in relation to flowering time of wheat, as measured by the control of stem rust and increase in yield, weight per bushel, and grade of Marquis wheat at Morris, Crookston, and St. Paul, Minnesota, in 1927*

Plot No.	Application			Stem Rust			Yield			Weight per bushel			Grade	
	Rate	No.	Time[a]	Mean Av.[b]	Decrease over check in		Mean average[b]	Increase over check in		Mean average[b]	Increase over check in		Mean average[b]	Increase over check in grades
					Actual	Proportional		Bu.	Per ct.		Lbs.	Per ct.		
56	15	3	FT + 6 + 4	26.1	39.5	60.2	31.62 ± 1.12	8.31	35.6	55.3	5.1	10.2	3	2
20	15	2	FT + 6	37.9	27.7	42.2	30.09 ± 1.06	6.78	29.1	55.2	5.0	10.0	3	2
1	15	1	FT	55.9	9.7	15.0	.....	.....	.....	.....	.....	.....	...	...
57	15	3	FT + 8 + 4	23.6	42.0	64.0	31.47 ± 1.11	8.16	35.0	56.0	5.8	11.6	3	2
21	15	2	FT + 8	40.0	25.6	39.0	30.31 ± 1.07	7.00	30.0	55.5	5.3	10.6	3	2
2	15	1	(FT + 5)	55.4	10.2	15.5	.....	.....	.....	.....	.....	.....	...	...
63	30	3	FT + 6 + 4	21.9	43.7	66.6	33.04 ± 1.17	9.73	41.7	56.1	5.9	11.8	3	2
24	30	2	FT + 6	38.4	27.2	41.5	31.41 ± 1.11	8.10	34.8	55.6	5.3	10.6	3	2
5	30	1	FT	55.0	10.6	16.2	.....	.....	.....	.....	.....	.....	...	...
64	30	3	FT + 8 + 4	17.6	48.0	73.2	34.35 ± 1.21	11.04	47.4	56.2	6.0	12.0	3	2
25	30	2	FT + 8	38.4	27.2	41.5	28.73 ± 1.01	5.42	23.3	55.6	5.4	10.8	3	2
6	30	1	(FT + 5)	56.7	8.9	13.6	.....	.....	.....	.....	.....	.....	...	...
Check	0	0	.....	65.6	.....	.....	23.31 ± 0.82	.....	.....	50.2	.....	.....	5	...
70	45	3	FT + 6 + 4	18.8	46.8	71.3	33.68 ± 1.19	10.37	44.5	56.2	6.0	12.0	3	2
29	45	2	FT + 6	52.1	13.5	20.6	31.53 ± 1.11	8.22	35.3	55.6	5.4	10.8	3	2
10	45	1	FT	57.1	8.5	13.0	.....	.....	.....	.....	.....	.....	...	...
71	45	3	FT +8 + 4	15.4	50.2	76.5	30.45 ± 1.08	7.14	30.6	55.8	5.6	11.2	3	2
30	45	2	FT + 8	38.4	27.2	41.5	32.57 ± 1.15	9.26	39.7	55.0	4.8	9.6	3	2
11	45	1	(FT + 5)	57.1	8.5	13.0	.....	.....	.....	.....	.....	.....	...	...
77	60	3	FT+ 6 + 4	14.0	51.6	78.7	36.27 ± 1.28	12.96	55.6	57.1	6.9	13.7	2	3
33	60	2	FT +6	38.4	27.2	41.5	29.61 ± 1.05	6.30	27.0	54.3	4.1	8.2	4	1
14	60	1	FT	50.4	15.2	23.2	29.06 ± 1.03	5.75	24.7	53.5	3.2	6.4	4	1
78	60	3	FT + 8 + 4	13.0	52.6	80.2	34.18 ± 1.21	10.87	46.6	57.1	6.9	13.7	2	3
34	60	2	FT + 8	32.6	33.0	50.3	30.21 ± 1.07	6.90	29.6	55.0	4.8	9.6	3	2
15	60	1	(FT + 5)	52.5	13.1	20.0	29.53 ± 1.04	6.22	26.7	53.1	2.9	5.8	4	1

[a] FT = One application at flowering time.
(FT + 5) = One application 5 days after flowering time.
FT + 6 = First application at flowering time and the second 6 days after the first.
FT + 8 = " " " " " " " " 8 " " " "
FT + 6 + 4 = First application at flowering time and the second 6 days after the first, with a third 4 days after the second.
FT + 8 + 4 = " " " " " " " " 8 " " " " " " " " 4 " " " "

[b] Mean average = Data from 12 plots, 4 systematically distributed plots at Morris, Crookston, and St. Paul.

compared with 50.2 pounds, the average of all the nondusted plots, represent increases of 3.3, 2.9, 2.4, and 3.3 pounds, respectively, as well as an increase in grade from No. 5 to No. 4. From the data at Crookston and St. Paul, whether considered separately or together, it is evident not only that the weight and grade of wheat for the plots dusted once are higher than those for the nondusted plots but also that the 15- and 30-pound rates were just as effective as the 45- and 60-pound rates.

A COMPARISON OF THE MEAN AVERAGE DATA OF THE RELATIVE EFFECTIVENESS OF THREE, TWO, AND ONE APPLICATION OF KOLO DUST APPLIED AT DIFFERENT RATES TO MARQUIS WHEAT

When comparing the data for the nondusted plots, the mean average proportional reductions in the amount of stem rust for plots dusted three times at the 15-, 30-, 45-, and 60-pound rates on the F.T. + 6 + 4 schedule were 60.2, 66.6, 71.3, and 78.7 per cent, respectively (Table 4, Plots 56, 63, 70, and 77); and for the F.T. + 8 + 4 schedule, they were 64.0, 73.2, 76.5, and 80.2 per cent, respectively (Table 4, Plots 57, 64, 71, and 78). For the plots dusted twice at the four rates on the F.T. + 6 schedule, the reductions were 42.2, 41.5, 20.6, and 41.5 per cent, respectively (Table 4, Plots 20, 24, 29, and 33); and for the F.T. + 8 schedule, they were 39.0, 41.5, 41.5, and 50.3 per cent, respectively (Table 4, Plots 21, 25, 30, and 34). For the plots dusted once at the four rates on the F.T. schedule, the reductions were 15.0, 16.2, 13.0, and 23.2 per cent, respectively (Table 4, Plots 1, 5, 10, and 14); and for the F.T. + 5 schedule, they were 15.5, 13.6, 13.0, and 20.0 per cent, respectively (Plots 2, 6, 11, and 15). From these data it is evident that two applications of Kolo dust were almost as effective as three and that the 15- and 30-pound rates were almost as effective as the 45- and 60-pound rates.

In comparing the data for the nondusted plots, the mean average increases in the yield of wheat for the plots dusted three times at the 15-, 30-, 45-, and 60-pound rates on the F.T. + 6 + 4 schedule were 35.6, 41.7, 44.5, and 55.6 per cent, respectively (Table 4, Plots 56, 63, 70, and 77); and for the F.T. + 8 + 4 schedule, they were 35.0, 47.4, 30.6, and 46.6 per cent, respectively (Table 4, Plots 57, 64, 71, and 78). For the plots dusted twice at the four rates on the F.T. + 6 schedule, the increases in yield were 29.1, 34.8, 35.3, and 27.0 per cent, respectively (Table 4, Plots 20, 24, 29, and 33); and for the F.T. + 8 schedule, they were 30.0, 23.3, 39.7, and 29.6 per cent, respectively (Table 4, Plots 21, 25, 30, and 34). Comparative data for plots dusted once at the 60-pound rate show increases of 24.7 and 26.7 per cent, respectively (Table 4, Plots 14 and 15). From these data it is evident that two applications were almost as effective as three and that the 15- and 30-pound rates were nearly as effective as the 45- and 60-pound rates.

When comparing the data for the nondusted plots, the mean average increases in weight per bushel for plots dusted three times at the 15-, 30-, 45-, and 60-pound rates on the F.T. + 6 + 4 schedule were 10.2, 11.8, 12.0, and 13.7 per cent, respectively (Table 4, Plots 56, 63, 70, and 77); and for the F.T. + 8 + 4 schedule, they were 11.6, 12.0, 11.2, and 13.7 per cent, respectively (Table 4, Plots 57, 64, 71, and 78). For the plots dusted twice at the four rates on the F.T. + 6 schedule, the increases in weight per bushel were 10.0, 10.6, 10.8, and 8.2 per cent, respectively (Table 4, Plots 20, 24, 29, and 33); and for the F.T. + 8 schedule they were 10.6, 10.8, 9.6, and 9.6 per cent, respectively (Table 4, Plots 21, 25, 30, and 34). Comparative data for plots dusted once at the 60-pound rate show increases of 6.4, and 5.8 per cent, respectively (Table 4, Plots 14 and 15). From these data it is evident that two applications of Kolo dust were almost as effective as three and, further, that the 15- and 30-pound rates were nearly as effective as the 45- and 60-pound rates.

Comparing the mean average data for the nondusted plots, the mean average increases in number of grade of the wheat from the plots dusted three times at the 15-, 30-, 45-, and 60-pound rates on the F.T. + 6 + 4 schedule were 2, 2, 2, and 3 grades, respectively (Table 4, Plots 56, 63, 70, and 77); and for the F.T. + 8 + 4 schedule, they were the same, being 2, 2, 2, and 3, respectively (Table 4, Plots 57, 64, 71, and 78). The increases in number of grade of wheat for plots dusted twice at the four rates for the F.T. + 6 schedule were 2, 2, 2, and 1 grades, respectively (Table 4, Plots 20, 24, 29, and 33); and for the F.T. + 8 schedule they were 2, 2, 2, and 2 grades, respectively (Table 4, Plots 21, 25, 30, and 34). Comparative data for the plots dusted once at the 60-pound rate show an increase of 1 grade each (Table 4, Plots 14 and 15). From these data it is evident that, although the increase in number of grade is not so great, two applications were nearly as effective as three and, further, that the 15- and 30-pound rates were practically as effective as the 45- and 60-pound rates.

THE EFFECT OF APPLYING KOLO DUST AT 30-, 45-, AND 60-POUND RATES, AT INTERVALS OF 3, 5, 7, 9, AND 11 DAYS, ON THE CONTROL OF STEM RUST, INCREASE IN YIELD, WEIGHT PER BUSHEL, AND GRADE OF MARQUIS WHEAT

Kolo dust was applied at the 30-, 45-, and 60-pound rates. Dusting was started at flowering time and subsequent applications were made at 3-, 5-, 7-, 9-, and 11-day intervals. The schedule and number of applications are shown on the next page.

The percentage of stem rust was lower on the dusted than on the nondusted plots, especially on those plots where the large number of applications were made at the shorter intervals (Table 5). The average per-

	Number of applications	*Intervals in days*
1.	8	3
2.	5	5
3.	4	7
4.	3	9
5.	3	11

centages of rust on the near-by nondusted plots were 67.5, 65.0, and 71.3, respectively (Table 5, Plots C–88, C–94, and C–100). The average percentages on the plots dusted at the 30-, 45-, and 60-pound rates at 11-day intervals (Table 5, Plots C–93, C–99, and C–105) were 24.5, 28.8, and 13.8, respectively, whereas on the plots dusted at the same rates at the 3-, 5-, 7-, and 9-day intervals, the rust averaged 10 per cent less. From these data it is evident that the 9-day interval is the upper limit for length of time between applications. The 30-pound rate appeared to be just as effective as the 45- and 60-pound rates in the control of rust.

The yields on some of the plots were quite valueless because of the unevenness of the stand of wheat in the portion of the field where this experiment was performed. Quack grass (*Agropyron repens*) was the principal cause of the unevenness. For this reason part of series C and D is omitted. The average yields were higher on the dusted than on the nondusted plots, especially where the large number of applications were made at the shorter intervals. The average yields of near-by nondusted plots were 32.40 ± 2.31, 33.25 ± 2.37, and 28.27 ± 2.01 bushels per acre, respectively (Table 5, Plots C–88, C–94, and C–100). The average yields on the plots dusted at the 30-, 45-, and 60-pound rates at the 11-day interval were 41.25 ± 2.94, 29.34 ± 2.09, and 39.87 ± 2.84 bushels per acre, respectively (Table 5, Plots C–93, C–99, and C–105); for the 9-day intervals, they were 48.76 ± 3.47, 33.61 ± 2.39, and 40.27 ± 2.87 bushels per acre, respectively (Table 5, Plots C–92, C–98, and C–104); at 7-day intervals, they were 48.14 ± 3.43, 44.33 ± 3.16, and 40.27 ± 2.87 bushels per acre, respectively (Table 5, Plots C–91, C–97, and C–103); at 5-day intervals, they were 46.10 ± 3.28, 48.24 ± 3.44, and 40.90 ± 2.91 bushels per acre, respectively (Table 5, Plots C–90, C–96, and C–102); and at 3-day intervals, the yields were still higher, being 48.72 ± 3.47, 49.61 ± 3.53, and 44.47 ± 3.17 bushels per acre, respectively (Table 5, Plots C–89, C–95, and C–101). From these data it is evident that the upper limit for time elapsing between applications is 9 days. It would appear that dusting at 3- or 5-day intervals, which would necessitate 8 and 5 applications, is not very practical, but that 4 applications at 9-day intervals, or possibly 3 applications at 8-day intervals starting at flowering time, would be just as effective, besides being far more practical.

TABLE 5.—*The effect of applications of Kolo dust when applied at the 30-, 45-, and 60-pound rates at intervals of 3, 5, 7, 9, and 11 days, on the control of stem rust, and on yield, weight per bushel, and grade of Marquis wheat at Crookston, Minnesota, in 1927*

Plot No.	Application			Stem rust per cent on the peduncle					Yield per acre in bushels					Weight per bushel in pounds					Average grade No.
	Rate in pounds per acre	No.	Time[a]	Series A	B	C	D	Av.	Series A	B	C	D	Average	Series A	B	C	D	Av.	
C– 88	0	0	Check	80	45	80	65	67.5	32.54	32.72	31.83	.....	32.40 ± 2.31	55.0	57.5	55.0	.....	55.8	3
C– 89	30	8	FT + 3 day intervals	tr	3	5	3	2.8	59.92	35.56	50.67	.....	48.72 ± 3.47	55.0	58.0	57.0	.....	56.7	2
C– 90	30	5	FT + 5 " "	tr	2	8	tr	2.5	65.61	38.23	46.58	33.96	46.10 ± 3.28	56.0	53.0	57.0	56.0	56.5	2
C– 91	30	4	FT + 7 " "	30	2	8	tr	10.0	48.18	42.85	49.25	52.27	48.14 ± 3.43	55.0	57.5	56.0	57.0	56.4	3
C– 92	30	3	FT + 9 " "	8	5	15	10	9.5	57.25	48.90	45.34	43.56	48.76 ± 3.47	56.0	57.5	55.0	57.0	56.4	3
C– 93	30	3	FT + 11 " "	18	30	25	25	24.5	44.81	44.09	35.56	40.54	41.25 ± 2.94	56.5	55.0	57.5	56.0	56.3	3
C– 94	0	0	Check	75	60	50	75	65.0	34.14	30.76	.....	34.85	33.25 ± 2.37	53.0	52.5	.....	54.0	53.2	4
C– 95	45	8	FT + 3 " "	10	tr	3	tr	3.3	46.23	62.76	40.89	48.54	49.61 ± 3.53	56.0	56.5	57.0	55.5	56.3	3
C– 96	45	5	FT + 5 " "	3	tr	3	2	2.0	49.78	40.72	.....	54.23	48.24 ± 3.44	54.0	56.5	.....	55.5	55.3	3
C– 97	45	4	FT + 7 " "	25	tr	3	2	7.5	42.32	21.34	.....	49.96	44.33 ± 3.16	55.0	56.5	.....	56.0	55.8	3
C– 98	45	3	FT + 9 " "	15	5	5	15	10.0	39.83	20.27	.....	40.72	33.61 ± 2.39	55.0	54.5	.....	55.0	54.8	3
C– 99	45	3	FT + 11 " "	45	10	45	15	28.8	29.16	19.56	34.14	34.49	29.34 ± 2.09	52.0	55.5	57.0	55.0	54.9	3
C–100	0	0	Check	85	30	85	85	71.3	27.91	23.65	30.40	31.12	28.27 ± 2.01	51.0	56.0	54.0	53.5	53.6	4
C–101	60	8	FT + 3 " "	tr	tr	2	tr	0.5	56.90	33.43	49.25	38.40	44.47 ± 3.17	58.0	58.0	56.0	58.0	57.5	1
C–102	60	5	FT + 5 " "	tr	5	2	2	2.3	32.18	34.14	56.90	40.36	40.90 ± 2.91	56.0	59.0	56.0	55.5	56.6	2
C–103	60	4	FT + 7 " "	5	5	3	3	4.0	42.85	38.05	43.56	36.63	40.27 ± 2.87	57.0	57.0	57.0	60.0	57.8	1
C–104	60	3	FT + 9 " "	10	5	10	tr	6.3	53.34	51.03	32.54	22.58	39.87 ± 2.84	58.0	59.0	57.0	58.0	58.0	1
C–105	60	3	FT + 11 " "	15	10	20	10	13.8	40.36	35.38	40.01	34.67	37.61 ± 2.68	54.5	59.0	57.0	59.0	57.4	2

[a] = FT + 3 day intervals = First application at blossom time with 7 subsequent applications at 3-day intervals.
FT + 5 " " " " " " " " 4 " " " " 5- " "
FT + 7 " " " " " " " " 3 " " " " 7- " "
FT + 9 " " " " " " " " 2 " " " " 9- " "
FT + 11 " " " " " " " " 2 " " " " 11- " "

Similar correlations of the control of stem rust and increase in yield with quality of wheat as measured by increases in weight per bushel and grade also were found. As might be expected, the wheat from the plots dusted at the shorter intervals, which necessitated a larger number of applications, weighed and graded higher than that from plots dusted at the longer intervals. As a rule the weight per bushel and grade were higher for the wheat from the dusted than that from the nondusted plots. The wheat from the near-by nondusted plots weighed 55.8, 53.2, and 53.6 pounds per bushel and graded No. 3, No. 4, and No. 4, respectively (Table 5, Plots C–88, C–94, and C–100). The wheat from the plots dusted at the 30-, 45-, and 60-pound rates at 3-day intervals weighed 56.7, 56.3, and 57.5 pounds per bushel and graded No. 2, No. 3, and No. 1, respectively (Table 5, Plots C–89, C–95, and C–101); for the 5-day intervals (Table 5, Plots C–90, C–96, and C–102), they were 56.5, 55.3, and 56.6 pounds per bushel and graded No. 2, No. 3, and No. 2, respectively; for the 7-day intervals (Table 5, Plots C–91, C–97, and C–103), they were 56.4, 55.8, and 57.8 pounds per bushel and graded No. 3, No. 3, and No. 1, respectively; for the 9-day intervals (Table 5, Plots C–92, C–98, and C–104), they were 56.4, 54.8, and 58.0 pounds per bushel and graded No. 3, No. 3, and No. 1, respectively; and for the plots dusted at the 11-day intervals (Table 5, Plots C–93, C–99, and C–105), they were 56.3, 54.9, and 57.4 pounds per bushel and graded No. 3 in the first two instances and No. 2 in the last. From these data it is evident that the wheat from the plots dusted at the 60-pound rate weighed and graded uniformly higher than that for the 30- and 45-pound rates.

Somewhat similar results also were obtained at Crookston when lodged Mindum wheat was dusted on the same schedule and at the same rates, although the control of stem rust was not so evident.

THE EFFECT OF NINE APPLICATIONS OF KOLO DUST WHEN APPLIED AT THE 60-POUND RATE BEFORE, DURING AND SUBSEQUENT TO FLOWERING TIME ON THE SETTING OF SEED, CONTROL OF STEM RUST, INCREASE IN YIELD, WEIGHT PER BUSHEL, AND GRADE OF MARQUIS WHEAT

Since flowering time was arbitrarily selected as the time to start dusting operations on wheat in Minnesota for the control of stem rust, it was thought that Kolo dust might inhibit or be injurious to seed setting, although such an effect was seriously doubted, because wheat is self-fertilized and therefore the possibility of Kolo dust gaining entrance to the florets prior to or at the time of anthesis would be very small. Consequently, Marquis wheat was dusted at the 60-pound rate daily for 6 days, starting 4 days before flowering time. Following these daily applications the plants were dusted three times at weekly intervals. The data obtained are summarized in table 6.

TABLE 6.—*The effect of 9 applications of Kolo dust when applied at the 60-pound rate before, during, and subsequent to flowering time of the wheat plant on the setting of seed and the control of stem rust, and on yield, weight per bushel, and grade of Marquis wheat at Crookston, Minnesota, in 1927*

Plot No.	Treatment	Average number of fertile flowers per spike	Stem rust percentage on peduncle Series					Yield per acre in bushels Series					Weight per bushel in lbs. Series					Average grade number
			A	B	C	D	Av.	A	B	C	D	Av.	A	B	C	D	Av.	
C– 0	Dusted	25.90	tr	tr	tr	tr	tr	65.61	65.61	63.83	.....	65.02	56.5	56.5	56.0	.....	56.3	3
C–82	Nondusted	22.06	95	70	85	80	82.5	26.14	27.74	40.72	35.20	32.45	52.0	56.0	55.4	54.0	54.25	4

In order to determine if there was any injurious effect to Kolo dust when applied before, during, and subsequent to flowering time, 100 spikes from the dusted and 100 from the nondusted plants were selected at random and the number of fertile florets per spike was recorded. It was found that there was no evidence of the failure of seed to set. In fact, the average number of fertile florets per spike was higher for the dusted than for the nondusted plots, being 25.90 and 22.06, respectively.

The dusted plants were almost completely free from stem rust. Only a trace was found in comparison with 82.5 per cent on the near-by nondusted plots.

The average yield of wheat from the plants dusted 9 times was 65.02 bushels per acre in comparison with 32.45 bushels per acre for that of the near-by nondusted plots. This represents slightly over a 100 per cent increase in yield for the dusted plants.

The average weight per bushel for the wheat from the plants dusted 9 times was 56.33 pounds and graded No. 3 in comparison with an average weight per bushel of 54.25 pounds and a grade of No. 4 for that of the near-by nondusted plots. Undoubtedly greater differences might have been found in the quality of wheat in the dusted and nondusted plots, if the former had been allowed to go to full maturity before being harvested. As it was, these dusted plots, still fairly green, were harvested along with the others which were then ripe.

THE EFFECT OF ONE APPLICATION OF KOLO DUST BY BROADCASTING OVER THE SOIL, ON THE CONTROL OF STEM RUST, INCREASE IN YIELD, WEIGHT PER BUSHEL, AND GRADE OF MARQUIS WHEAT

It was thought that there might be some fertilizer effect of Kolo dust that would not be taken into consideration when interpreting the data. Consequently, an experiment was conducted in which Kolo dust was applied broadcast to the soil between the rows of wheat at flowering time, at the following pound rates per acre: 30, 60, 120, 240, 480, 960, and 1,920. The data are summarized in table 7.

There was no observable evidence of the control of stem rust on the plots to which Kolo dust was applied broadcast to the soil when compared with that on the plots to which no Kolo dust was applied. The average percentage of stem rust on the peduncle of the plants in this experiment ranged from 53.8 to 61.3. If it is assumed that the volatile sulphur fumes in Kolo dust are a toxic agent, then there apparently was an insufficient amount of sulphur volatilized at any one time from Kolo dust on the soil to inhibit the development of the rust fungus.

There appeared to be no fertilizing effect by broadcasting Kolo dust to the soil. The average yields of wheat on the plots to which Kolo dust had been applied broadcast were not significantly different from those on the plots to which no Kolo dust had been applied.

TABLE 7.—*The effect of Kolo dust applied broadcast to the soil on the control of stem rust and on yield, weight per bushel, and grade of Marquis wheat sown late at St. Paul, Minnesota, in 1927*

Plot No.	Rate per acre of application in pounds	Stem rust per cent on the peduncle					Yield per acre in bushels					Average weight per bushel in pounds	Average grade number
		Series				Av.	Series				Average		
		A	B	C	D		A	B	C	D			
UF– 165	30	45	65	60	65	58.8	7.56	5.04	6.84	13.34	8.15 ± 0.81	46.0	Sample grade
UF– 166	60	40	65	60	60	56.3	10.85	10.31	12.80	12.98	11.74 ± 1.17	45.5	" "
UF– 167	120	45	55	65	60	56.3	12.98	5.40	12.45	11.91	10.69 ± 1.07	47.5	" "
UF– 168	240	65	65	60	55	61.3	9.42	6.48	12.09	17.78	11.44 ± 1.14	48.0	" "
UF– 169	0	95	45	55	55	62.5	7.70	11.56	11.20	14.22	11.05 ± 1.10	45.5	" "
UF– 170	480	60	45	60	50	53.8	6.84	7.20	11.20	14.94	10.05 ± 1.00	45.0	" "
UF– 171	960	50	50	60	60	55.0	8.89	10.13	11.73	12.62	10.89 ± 1.09	45.0	" "
UF– 172	1920	60	40	60	60	55.0	2.70	10.31	12.27	13.69	9.74 ± 0.97	47.0	" "
UF– 173	0	55	50	65	65	58.8	5.40	3.60	16.18	8.89	8.52 ± 0.85	46.5	" "

Similarly, the weights per bushel and grades of wheat from the plots to which Kolo dust was applied broadcast did not differ significantly from those of the plots to which no Kolo dust was applied.

A similar experiment was carried out at Crookston where Kolo dust was applied broadcast to the soil, at the same rate and time, between the rows of Mindum wheat. There were no observable evidences of the control of stem rust, nor increases in yield, weight per bushel, or grade of wheat from such treated plots.

THE EFFECT OF SEVEN APPLICATIONS OF KOLO DUST WHEN APPLIED AT THE 60-POUND RATE AT 5-DAY INTERVALS ON THE CONTROL OF ORANGE LEAF AND STEM RUSTS, INCREASE IN YIELD, WEIGHT PER BUSHEL, GRADE, AND PROTEIN CONTENT OF RUBY WHEAT

Ruby wheat was sown in three 1/40-acre plots on April 27. One-half of each plot was dusted with Kolo dust at the 60-pound rate. The first application was made on June 27, one week before flowering time, and 6 subsequent applications were applied at 5-day intervals. Notes on the prevalence of orange leaf rust were taken on August 1 and on stem rust on August 8. Three separate square-yard samples were harvested from both the dusted and nondusted portions of each of the 1/40-acre plots on August 8. The data obtained are summarized in table 8.

Since very little stem rust developed at Crookston on Ruby wheat, which was 10 days earlier than Marquis, there were no significant differences in the amount of stem rust on the peduncles of the dusted and nondusted plants. However, there were significant differences in the amount of orange leaf rust on the dusted and nondusted plants. On the former there was only 26.7 per cent in comparison with 97 per cent for the latter. The leaves withered and dried up on the nondusted plants, whereas the leaves on the dusted plants remained green and functioned for a longer time.

The average yield of wheat from the dusted plants was 28.65 bushels per acre in comparison with 21.00 bushels per acre for the nondusted plants. This represents a 7.65 bushel or a 26.7 per cent increase in yield as a result of the reduction in the amount of orange leaf rust and what little reduction there was in the amount of stem rust.

The average weight per bushel for the wheat from the dusted plants was 61.0 pounds in comparison with 56.7 pounds for that of the nondusted plants. This represents an increase of 4.3 pounds per bushel in weight for the wheat from the dusted plants. The wheat from the dusted plants graded No. 1 in comparison with a grade of No. 2 for that of the nondusted plants. As a further indication of the shrinking of the wheat kernels, 250

TABLE 8.—*The effect of 7 applications of Kolo dust when applied at the rate of 60 pounds per acre at 5-day intervals on the control of orange leaf and stem rusts and on yield, weight per bushel, grade, and protein content of Ruby wheat at Crookston, Minnesota, in 1927*

Plot no.	Treatment	Series			Average
		A	B	C	
		Stem rust per cent on the peduncle			
C–400	Dusted	2.00	1.00	3.00	2.00
C–401	Nondusted	4.00	5.00	4.00	4.30
		Leaf rust per cent			
C–400	Dusted	25.00	30.00	25.00	26.70
C–401	Nondusted	98.00	98.00	95.00	97.00
		Yield per acre in bushels			
C–400	Dusted	31.17	28.80	25.96	28.65
C–401	Nondusted	23.47	19.79	19.74	21.00
		Weight per bushel in pounds			
C–400	Dusted	61.00	61.00	61.00	61.00
C–401	Nondusted	57.00	56.00	57.00	56.70
		Weight per 250 seeds in grams			
C–400	Dusted	2.99	2.96	2.88	2.94
C–401	Nondusted	2.37	2.08	2.23	2.23
		Protein content in per cent			
C–400	Dusted	15.88	15.38	13.13	14.80
C–401	Nondusted	14.80	15.44	15.25	15.16
		Grade number			
C–400	Dusted	1	1	1	1
C–401	Nondusted	2	3	2	2

seeds were picked at random from each lot and weighed. The 250 seeds from the dusted plants weighed 0.718 gram more than seeds from the nondusted plants. Undoubtedly orange leaf rust had some effect on the shrinking of the wheat kernels.

The protein content of the wheat from the nondusted was slightly higher than that from the dusted plants. The average protein-content percentage was 15.16 for the former in comparison with 14.80 for that of the latter. Dusting with Kolo dust to control orange leaf rust and stem rust hardly would be expected to affect the protein content of wheat. The wheat from

the nondusted plants was more shrunken and the relative amounts of carbohydrates and water are probably lower than in the plumper wheat from the dusted plants. Consequently, since the protein content is not affected by the rust, it would be proportionately higher in the wheat from the nondusted plants than in that from the dusted plants.

SUMMARY

1. Experiments on the control of cereal rusts by dusting with Kolo dust were carried out at Morris, Crookston, and St. Paul, Minnesota, in 1927. Varieties of wheat most extensively grown in Minnesota were used in these tests. Marquis and Ruby, common bread wheats, and Mindum, a durum wheat, were grown. There were over 2,000 plots in the entire experiment at these three stations.

2. The mean averages of the data on the Marquis wheat plots at Morris, Crookston, and St. Paul, dusted 3 times with Kolo dust, show as high as an 80.2 per cent reduction in the amount of stem rust; a 57.1 per cent increase in yield; and a 13.7 per cent increase in weight per bushel, as well as an increase in the grade of wheat when compared with that of the nondusted plots. The most effective dusting schedule for three applications of Kolo dust at the three stations from the standpoint of timeliness of application was the F.T. + 6 + 4, or F.T. + 8 + 4 schedule, where the second application was made either 6 or 8 days after the flowering-time application followed by a third application 4 days after the second. The 15- and 30-pound rates were found to be essentially as effective as the 45- and 60-pound rates.

3. The mean averages of the data on the Marquis plots dusted twice with Kolo dust show as high as 50.3 per cent decrease in the amount of stem rust; a 39.7 per cent increase in yield; and a 10.8 per cent increase in weight per bushel, as well as an increase in the grade of wheat when compared with that of the nondusted plots. The most effective schedule for two applications of Kolo dust at the three stations was the F.T. + 6, or F.T. + 8 schedule, where the second application was made either 6 or 8 days after the flowering-time application. Again the 15- and 30-pound rates were found to be nearly as effective as the 45- and 60-pound rates.

4. Although with two applications of Kolo dust the control of stem rust was not so great as with three applications, nor the increase in yield, weight per bushel, and grade so high, yet for practical purposes two applications of Kolo dust were almost as effective as three, and further the 15-pound rate was practically as effective as the 60-pound rate.

5. Although there were no significant observable evidences of the control of stem rust with one application of Kolo dust, such treated plots repeatedly yielded slightly more wheat which weighed and graded slightly higher than that for the nondusted plots.

6. At Crookston, when Marquis wheat plots were dusted with Kolo dust at the 30-, 45-, and 60-pound rates at 3-, 5-, 7-, 9-, and 11-day intervals starting at flowering time, the upper limit for length of time elapsing between applications of Kolo dust was found to be 9 days. It would appear that dusting at 5- or 3-day intervals, which would necessitate five or eight applications, is not very practical. Four applications at 7-day intervals or three applications at 8- or 9-day intervals starting at flowering time, which were almost as effective as the larger number of applications at shorter intervals, would be far more practical.

7. At Crookston, Marquis wheat plots dusted with Kolo dust at the 60-pound rate before, during, and subsequent to flowering time of the wheat plant did not inhibit or prevent the setting of seed. No injury whatsoever was observed.

8. At St. Paul, when Kolo dust was applied broadcast to the soil between the rows of Marquis at flowering time in amounts ranging from 30 to 1,920 pounds per acre, there were no observable evidences of the control of stem rust, nor increases in yield, weight per bushel, and grade of wheat from such treated plots in comparison with those for the plots to which no Kolo dust was applied. Similar results also were obtained at Crookston where Kolo dust was applied broadcast to the soil at the same rate and time in a field of Mindum wheat.

9. At Crookston, Ruby wheat plants dusted seven times with Kolo dust at the 60-pound rate at 5-day intervals yielded 26.7 per cent more wheat than nondusted plants. This increase in yield, as well as an increase in weight per bushel and grade, was ascribed to the marked decrease in the amount of orange leaf rust, since there was very little stem rust in the Ruby plots. The protein content of wheat from the nondusted plants was found to be slightly higher than that of the dusted plants, being 15.16 and 14.80 per cent, respectively.

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CROSS-INOCULATION EXPERIMENTS WITH ERIGERON YELLOWS AND PEACH ROSETTE

J. A. McCLINTOCK

Reference to "The Plant Disease Reporter"[1, 2, 3, 4, 5, 6, 7, 8] indicates that peach rosette is on the increase and is spreading farther northward. Its reported presence in Kentucky and Illinois within the past three years indicates that this disease has wild-host plants in these new areas and that natural carriers are spreading it to cultivated hosts of commercial importance.

As early as 1891 Smith (11) suspected that rosette-infected wild plums were a natural source of infection for peaches, and later the writer (10) verified Smith's suspicions.

Failure to add to the contributions regarding the natural means of disseminating peach rosette led the writer to consider the suggestion by Valleau[3] that diseased wild Erigeron plants might be associated with the presence of peach rosette.

According to "The Plant Disease Reporter,"[2] peach rosette was reported from Tennessee by Waite, in 1907, by Essary, in 1913 and 1917, and by Hesler, in 1920, but no mention was made of its association with wild hosts in that State. Since 1922 the writer has observed natural infections of rosette in peaches and plums in Maury, Williamson, Madison, and Knox counties, Tenn. Search in the vicinity of diseased trees in each case failed to locate rosetted wild plums from which such infections might have spread.

Erigeron canadensis L. is a common weed in waste lands and abandoned fields throughout Tennessee. Yellows-infected Erigeron plants are not

[1] [Valleau, W. D.] Peach rosette found in Kentucky. U. S. Dept. Agr., Bur. Plant Industry. Plant Dis. Rptr. 11: 133. 1927. [Mimeographed.]

[2] United States Department of Agriculture, Bureau of Plant Industry. Peach rosette. Plant Dis. Rptr. Sup. 60: 170–171. 1928. [Mimeographed.]

[3] [Valleau, W. D.] Outbreak of peach rosette. U. S. Dept. Agr., Bur. Plant Industry. Plant Dis. Rptr. 12: 62–63. 1928. [Mimeographed.]

[4] [Anderson, H. W.] A report of peach rosette from Illinois. U. S. Dept. Agr., Bur. Plant Industry. Plant Dis. Rptr. 12: 90. 1928. [Mimeographed.]

[5] United States Department of Agriculture, Bureau of Plant Industry. Reports on peach yellows, rosette and little peach. Plant. Dis. Rptr. 12: 103. 1928. [Mimeographed.]

[6] [Waite, M. B.] Peach rosette in South Carolina. U. S. Dept. Agr., Bur. Plant Industry. Plant Dis. Rptr. 12: 142. 1928. [Mimeographed.]

[7] United States Department of Agriculture, Bureau of Plant Industry. Peach rosette. Plant Dis. Rptr. Sup. 70: 219. 1929. [Mimeographed.]

[8] [Waite, M. B.] Peach rosette occurs in Oklahoma. U. S. Dept. Agr., Bur. Plant Industry. Plant Dis. Rptr. 13: 52. 1929. [Mimeographed.]

uncommon wherever this weed grows, and a study of these diseased plants in various sections of Tennessee indicates that they have symptoms strikingly similar to rosette of peach.

Experiments by Kunkel (6) have proved that yellows of China asters, *Callistephus chinensis* Nees, can be transmitted to *Erigeron annuus* L. and *E. canadensis* L.; therefore, it is assumed that in using the diseased wild Erigeron plants the writer was working with the aster-yellows virus. *Erigeron canadensis* is the only species of Erigeron used in these experiments.

Cross-inoculation experiments with aster yellows and peach yellows conducted by Kunkel (6) led the writer to suspect that yellows in asters and its other host plants was different from the various virus diseases of the peach. However, the suggestion by Valleau[9] brought to attention the fact that peach rosette is distinct from peach yellows and has a greater known host range and might, therefore, be transmitted to Erigeron with symptoms similar to aster yellows and yet appear in peach and plum as a rosette disease.

As the control of these virus diseases is closely associated with a knowledge of their wild as well as their cultivated hosts, it seemed desirable to study the relation between Erigeron yellows and peach rosette.

In *Erigeron canadensis* the causal agent of yellows is virulent, like the causal agent of rosette in peach, often killing the host quickly, as contrasted with mosaics in other annuals and yellows in peaches. This is illustrated

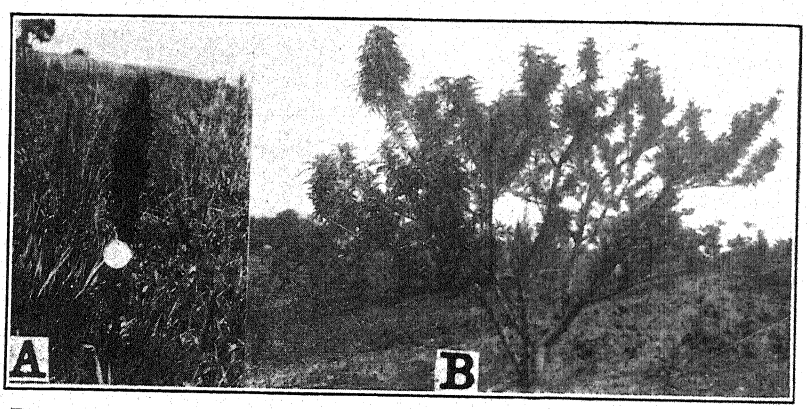


FIG. 1. A. A plant of *Erigeron canadensis* which became naturally infected with aster yellows early in the season. This plant attained a height of 16½ inches and died by August 5, 1930, without producing any seed. B. A peach tree completely infected with rosette. Note the greatly shortened internodal growth. Compare with A and figure 2, A.

[9] *Loc. cit.*, 3.

by figure 1, A, a natural infection of Erigeron which was first observed June 18, 1930, and was entirely dead when observed August 5, 1930. Numerous similar cases were observed during 1929. Reference to figure 1, A, also shows another similarity between Erigeron yellows and peach rosette (Fig. 1, B), namely, the greatly shortened internodal growth, which produces a rosetted appearance in both cases. This Erigeron plant, infected early in the season, attained a height of only 16½ inches. While in contrast numerous healthy plants of *E. canadensis* attained a height of 6 to 8 feet before being killed by cold weather in the late fall of 1929.

In cases where Erigeron plants attain considerable size before being infected, only the new growth shows the typical yellows appearance (Fig. 2, A). The same is true of peaches and plums that become infected with peach rosette after considerable growth has been made during a given growing season. *Erigeron canadensis* may also have the one-sided type of infection described by Kunkel (6) in asters. This is also true of peaches and plums in cases of early rosette infection. Peach trees infected by rosette

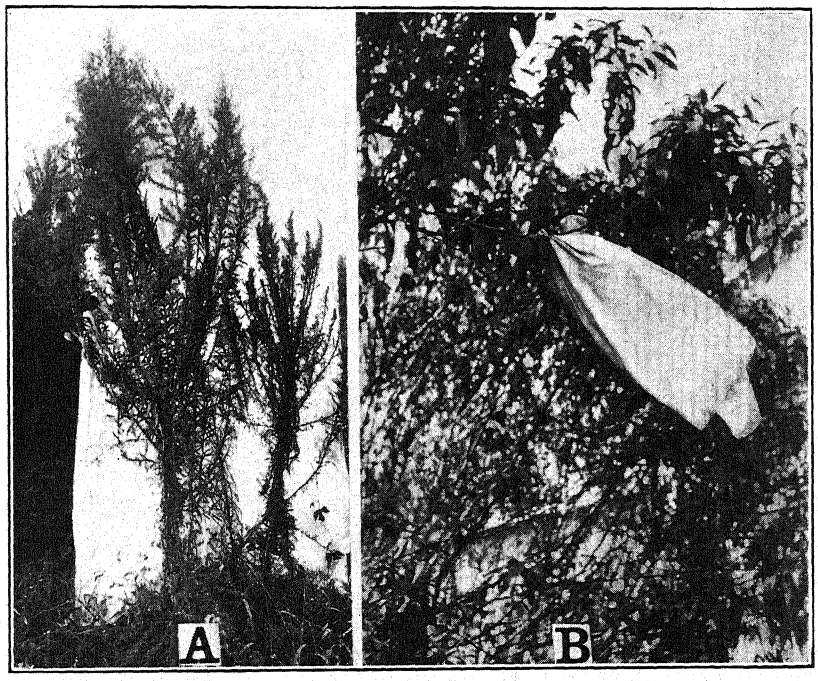


FIG. 2. A. Left, a healthy plant of *Erigeron canadensis*. Right, a similar plant which became infected with aster yellows after it had attained considerable size. Note the shortened internodal development on all of the new top growth of this plant. B. A peach limb with attached cloth bag used as a cage to hold yellows-infected Erigeron plant and its associated insects in close contact with tender peach shoots and leaves.

develop a pale yellowish green color, in contrast to the normal green of healthy peach trees. This is strikingly the case in *E. canadensis* infected with yellows.

Peach trees that become infected with rosette after they have set fruit may mature that crop, yet trees that show a complete rosetted condition early in the spring generally fail to set any fruit, even though they have produced blossoms. Reference to figure 1, A, shows that early natural infection of *Erigeron canadensis* with yellows also results in the failure of the plant to produce seed. Numerous cases of this kind were observed in 1929.

EXPERIMENTS ON SEED TRANSMISSION OF ERIGERON YELLOWS

Reddick and Stewart (10), McClintock (7), Gardner and Kendrick (5), Dickson (2), Archibald (1), and Fajardo (4) have found that the seed of various mosaic-infected leguminous plants transmitted the causal agent; while in the composite plants Kunkel (6) has proved that yellows-infected asters did not transmit the causal agent through the seed. Kunkel states, however, "It is possible that yellows may be transmitted through the seeds of some host plant other than asters." As the work of Doolittle and Walker (3) showed that the seed of the wild cucumber (*Micrampelis lobata* (Michx.) transmits the causal agent of cucumber mosaic, while the seeds of cultivated cucumbers, muskmelons, pumpkin, and squash do not, it seemed desirable to test the seed of *Erigeron canadensis* as one of the wild hosts of aster yellows.

Under the mild weather conditions of Tennessee, *Erigeron canadensis* is often a winter annual. The seeds germinate and produce small plants which grow slowly throughout the fall and winter and complete their growth during the following spring and summer. Thirty-four such plants, dug at random over the University farm and brought to the greenhouse December 13, 1929, developed no symptoms of yellows, and in August, 1930, matured seed the same as seedlings started in flats in the greenhouse.

Plants of *Erigeron canadensis* that become infected with yellows after making considerable growth may mature some seed. During the summer and fall of 1929 mature seed was collected from several typical yellows-infected Erigeron plants growing on the University farm. On November 2, 1929, seed from two of these diseased plants was planted in two flats of good greenhouse soil and held on a greenhouse bench. This seed was somewhat slower in germinating than seed from healthy plants planted in other flats on the same date, but the hundreds of seedlings from the two yellows-infected Erigeron plants grew normally and never developed any symptoms of disease. These plants were not transplanted, but during August, 1930, the few plants left in the greenhouse were producing seed the same as any other healthy plants.

On May 22, 1930, another flat was thickly planted to seed collected from one yellows-infected Erigeron plant on August 6, 1929. In this case the seedlings were above ground in 8 days, the same as those from seed of healthy plants seeded on the same date. Both lots of seedlings were allowed to grow in their respective flats on greenhouse benches until August 14 when they were transplanted to 4-inch pots. None of the 378 plants from seed collected from the yellows-infected Erigeron plants showed any symptoms of yellows during this 84-day period.

These tests indicate that seed from plants of *Erigeron canadensis* infected with yellows do not transmit the causal agent to their seedlings and that Erigeron seeds are probably not a factor in disseminating yellows.

EXPERIMENTS ON INSECT TRANSMISSION FROM YELLOWS-INFECTED ERIGERON TO PEACH

Observations of both healthy and diseased plants of *Erigeron canadensis* in various stages of development disclosed the fact that they are frequented by various insects, including numerous unidentified beetles and grasshoppers; tarnished plant bugs, *Lygus pratensis* Linnaeus; several leaf hoppers, as *Empoasca mali* LeBaron, *Empoasca flavescens* Fab., and *Cicadula sexnotata* Fall; and some aphids, as the green peach aphid, *Rhopalosiphum persicae* Sulzer, the green potato aphid, *Macrosiphum solanifolii* Ashmead; the potato flea beetle, *Epitrix cucumeris* Paris; and the twelve-spotted cucumber beetle, *Diabrotica duodecimpunctata* Olivier. The bunching of the leaves on the yellows-infected Erigeron plants furnished good hiding places for insects, and it was surprising how many could be found on a single diseased plant.

To obtain material for mass insect transfers, the writer used new porous-cloth bags with a draw string at the top and a cloth label sewed in at the bottom. These bags, measuring about 17 inches in length and 7 inches in diameter, when fully opened, were taken to the field to serve as insect cages. By selection among the yellows-infected plants of *Erigeron canadensis,* typical ones were obtained that were a size suitable to enclose within a bag. By drawing an open bag down over the top of a plant, then breaking off the stem and quickly pulling the draw string, it was possible to cage most of the insects on a diseased plant. The insects on 10 typical yellows-infected Erigeron plants were thus caged in separate bags on August 6, 1929. These were carried at once to a row of healthy peach trees, where, one by one, the bags were carefully opened and slipped over the ends of peach limbs. The draw strings were then wrapped several times around the closed mouth of each bag and tied, thus holding the enclosed yellows-infected Erigeron tops and the associated insects in close contact with tender growing peach shoots and leaves, as seen in figure 2, B.

On August 14, or 9 days after the 10 bags were tied about the peach limbs, 5 were loosened and removed. The peach limbs and leaves showed no ill effects from enclosure in the porous-cloth bags, but the Erigeron tops were wilted and dry. Various insects were still alive; therefore, they must have fed on the enclosed peach leaves after the Erigeron tops had dried. The other 5 bags were left closed about the peach shoots throughout the fall, winter, and spring. When these bags were removed, May 1, 1930, the portions of the limbs that had been within the bags were dead or dying; while the rest of each limb appeared normal and fruits had set the same as on other limbs. At this time each of the 10 trees upon which bags of insects had been caged was carefully examined and found to be normal and bearing a good crop of fruit. These 10 trees were under observation throughout the summer and have matured normal crops of fruit. Up to September 1, 1930, none has developed symptoms of peach rosette, or any other symptoms that might be suspected of coming from infection of diseased Erigeron plants.

As the leaf hopper, *Cicadula sexnotata*, which Kunkel (6) has proved to be a carrier of aster yellows to Erigeron and back to asters, was present, along with other insects on the yellows-infected Erigeron plants enclosed in the bags about the peach limbs, opportunity was afforded for the transfer of the causal agent from diseased Erigeron to peach. The fact that no disease appeared on the 10 peach trees indicates that Erigeron yellows is not readily transmitted to peach and probably is a different disease from peach rosette.

EXPERIMENTS ON MECHANICAL TRANSMISSION OF ERIGERON YELLOWS TO PEACH

On August 6, 1929, a number of Erigeron plants in various stages of yellows were pulled up, root and all, and run through a grinder. The crushed mass was then put in a press and 130 cc. of juice extracted and enclosed at once in a tightly corked bottle. By means of a large hypodermic needle with a fine point, tap-water was injected into a number of peach twigs and fruits to serve as checks. With the same needle, undiluted juice extracted from the yellows-infected Erigeron plants was injected into more than 100 growing peach tips and green fruits. These inoculations included 20 peach trees. No disease developed during the remainder of the season of 1929, and in the spring of 1930 these trees blossomed, foliated, and later bore a crop of normal fruit, the same as the check trees. Up to September 1, 1930, none of these trees showed any symptoms of disease.

These experiments indicate that juice from yellows-infected Erigeron plants will not readily produce rosette when artificially injected into actively growing peach shoots and fruits.

EXPERIMENTS ON THE TRANSMISSION OF ROSETTE FROM PLUM TO PEACH AND CHERRY BY BUDS

To determine the relative effectiveness of Erigeron yellows, and true rosette of Prunus in transmitting rosette, buds were taken from a typical rosetted plum tree on August 14, 1929, and put in 4 trees of cultivated varieties of peaches and 4 sand cherries, *Prunus pumila* L. In all cases bark buds were inserted and tied with $\frac{1}{4}$-inch rubber strips manufactured for nursery purposes. The buds all united with the respective stocks, but no symptoms of rosette appeared during the rest of the 1929 growing season.

On December 7, 1929, 2 of the 4 sand cherries into which rosette plum buds were inserted were dug from the nursery row and set in 2 10-inch

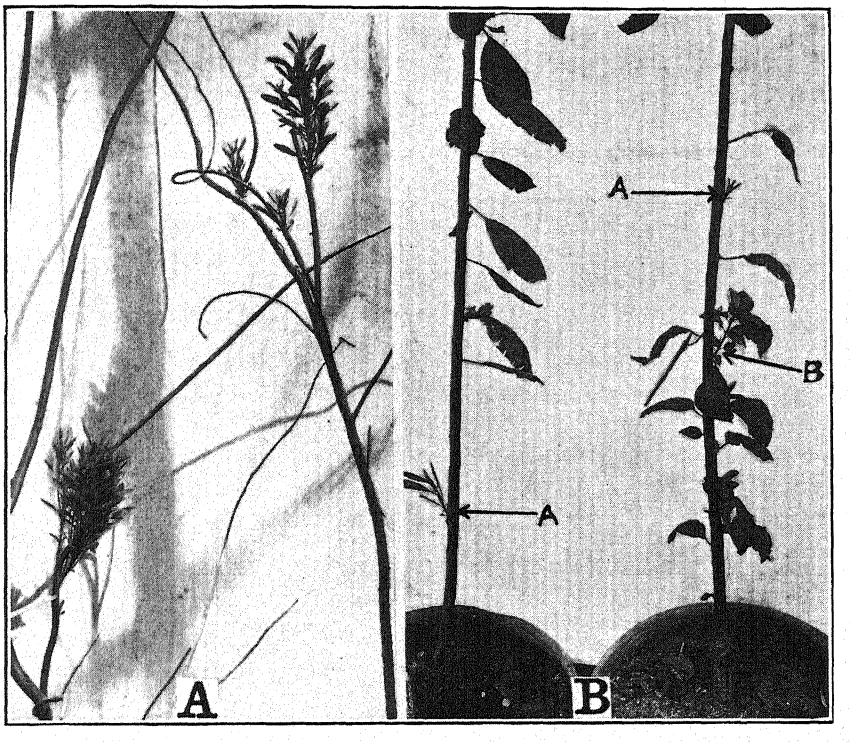


FIG. 3. A. Sand cherries inoculated with buds from a rosette-infected plum. Note the typical rosetted cherry shoots from the few lateral buds which developed. The balance of the lateral, and the terminal buds are still dormant. These 2 sand cherries were the source of inoculum for cross inoculations to Erigeron and plum. B. Plum trees into which dormant buds from rosette sand cherries were inserted April 2, 1930. Note the condition August 26, 1930, with rosette sand-cherry shoots at A and rosette plum shoots at B. Other rosette plum shoots are partly covered by older leaves.

pots, which were transferred to the greenhouse. On February 6, 1930, symptoms of rosette began to appear on the new growth of both sand cherries growing in the greenhouse. The manifestations of rosette on these 2 sand cherries differed from rosette on this host under field conditions in that only a few lateral buds grew into rosetted shoots early in February, while most of the lateral and some of the terminal buds remained dormant, as seen in figure 3, A. Some weeks later additional lateral and terminal buds developed rosetted shoots. In all cases the new growth was typically rosetted, and the few blossoms which opened failed to set fruit. These 2 sand cherries were the source of all rosetted material used in subsequent tests in the greenhouse unless otherwise stated.

With the beginning of new growth in the spring of 1930 the other 2 sand cherries developed typical rosette in all of their new growth. The 4 peach trees budded to rosette plums August 14, 1929, also developed typical rosette in the spring of 1930. Thus rosette was transferred by budding from rosette plum to peach and sand cherry in 100 per cent of the cases, while Erigeron-yellows inoculations made about the same time were entirely negative.

These experiments indicate that conditions were favorable for infection of rosette at the time the Erigeron-yellows inoculations were made in the field during the summer of 1929. This is further evidence that Erigeron yellows and peach rosette are two different diseases.

Incidentally, this is the first record of rosette being artificially transmitted to sand cherries, though one case of natural infection of sand cherries with rosette was observed by the writer during the summer of 1929.

As the pits from which these sand cherries were raised came from large, wild thickets on the sand dunes near Lake Michigan, the presence of rosette in Illinois, as previously mentioned, indicates that this disease is getting near an abundant supply of wild host plants in the North.

EXPERIMENTS ON MECHANICAL TRANSMISSION OF ROSETTE FROM SAND CHERRIES TO ERIGERON CANADENSIS

On February 22, 1930, 4 healthy plants of *Erigeron canadensis* were inoculated with rosette as follows. Leaves were cut from the 2 rosetted sand cherry trees growing in the greenhouse, and the leaf blades and petioles crushed into leaf blades and petioles of the Erigeron plants. The plants were then labeled and at once placed under closed bell-jars. Under these humid conditions the rosetted sand cherry leaves dried very little, but within a few days fungi began to develop on the crushed surfaces, and the bell-jars were therefore removed. The Erigeron plants continued to grow normally on the greenhouse benches. By May 9, 1930, these plants were about 18 inches tall and appeared as normal as check plants which

had had only tap-water crushed into their leaves and petioles on February 22. On August 12, 1930, both the inoculated plants and checks had matured seed without showing any symptoms of disease.

On March 13, 1930, a succulent shoot from a rosette sand cherry was crushed into the tips of each of the new leaves of a healthy potted Erigeron plant and the plant at once placed under a low closed bell jar and left until March 15. The film of moisture that collected on the under side of the bell jar indicated that the air inside of the jar was very humid during this 3-day period. By August 12, 1930, this plant and its check had each matured a crop of seed without either showing any symptoms of disease.

On March 13 exudate from the leaf glands of rosetted sand cherries was transferred on a flamed dissecting needle and pricked into the leaf blades and petioles of a potted Erigeron plant. Tap water pricked into a similar plant with a flamed needle served as a check. The plants were placed under closed bell jars for several days and then held on the greenhouse bench. By May 9 these plants had reached a height of 10 inches and both appeared healthy. By August 12 these plants had matured crops of seed without showing any symptoms of disease. These experiments indicate that the causal agent of rosette cannot be readily transmitted from sand cherries to plants of *Erigeron canadensis* by the ordinary mechanical methods used in transferring virus diseases of the mosaic type in other plants.

On May 17, 1930, two bark buds were cut from 1930 rosette growth of sand cherries and put in T-shape slits through the bark of 2 plants of *Erigeron canadensis* 18 inches tall, growing in 6-inch pots. At the same time 4 wood buds were cut from 1-year-old growth of a plum that had shown typical rosette throughout the season of 1929. These buds were set in T-shape slits through the bark of 4 Erigeron plants 15 to 18 inches tall, growing in 6-inch pots. Two strips of bark without buds were cut from 1-year-old shoots of the same rosetted plum and put into slits through the bark of 2 Erigeron plants 16 inches tall, growing in 6-inch pots. All of these buds and bark strips, as well as checks, consisting of healthy buds and bark strips similarly inserted, were tied with $\frac{1}{4}$-inch rubber strips. There was no expectation that tissue union would take place between the sand cherries or plums and the Erigeron plants, but it was known that this method would hold rosette Prunus tissues in close contact with succulent Erigeron stem tissues. Both the inoculated plants and the checks continued to grow normally on the greenhouse bench and by August 12 were producing flowers and seed without showing any symptoms of disease. These experiments further indicate that rosette of species of Prunus is not readily transmitted to plants of *E. canadensis* by mechanical means. Together

with the other experiments, this failure of mechanical transfer indicates that peach rosette is not the same as Erigeron yellows.

EXPERIMENTS ON INSECT TRANSMISSION OF PEACH ROSETTE FROM SAND CHERRIES TO ERIGERON

As these experiments were conducted in limited greenhouse space, the writer had to rely on such insects as chanced to be available.

In experiments with spinach blight McClintock and Smith (8), and, later, in studies on peach rosette, McClintock (9) observed that feeding aphids that were quickly or roughly removed from plants on which they were feeding were often injured so that they did not thrive when transferred to a new host. If similar feeding aphids were disturbed by being brushed lightly with a small camel's-hair brush they usually ceased feeding and began to crawl about on the host plant. They could then be removed with the brush and, when transferred to a new host, would generally quiet down and resume feeding in a short time. This method was used in all of the following greenhouse transfers of aphids and appeared to be successful except in the case of the melon aphid, as mentioned later.

TRANSMISSION EXPERIMENTS WITH GREEN PEACH APHIDS

On March 13, 1930, a number of young and mature green peach aphids, *Rhopalosiphum persicae* Sulzer, that had been feeding for more than a week on succulent, rosette sand-cherry shoots in the greenhouse, were removed to a half Petri dish with a camel's-hair brush. The aphids were brushed from the dish and allowed to fall onto the leaves of several potted Erigeron plants. After labeling, the pots were held on the greenhouse bench for several days; then the aphids were killed by spraying with a commercial brand of pyrethrum soap. The plants continued to grow normally and, early in August, matured a crop of seed without showing any symptoms of disease.

On March 13, 6 adult green peach aphids which had been feeding for more than a week on rosette sand-cherry shoots were removed with a camel's-hair brush, and 3 were transferred to each of 2 healthy potted Erigeron plants. After labeling, the pots were enclosed in a bell jar for several days. The aphids were then killed with a pyrethrum soap spray. By May 9 the 2 plants had reached a height of 8 and 12 inches, respectively, and showed no symptoms of disease. By August 12 both plants were nearing maturity and appeared as normal as the check plants.

On March 17, an adult green peach aphid, which had fed more than a week on rosette sand cherry tissue, was transferred by a camel's-hair brush and dropped onto a healthy Erigeron plant. During the several days this plant was held under a bell jar, a colony of young aphids became well estab-

lished on it. About two weeks later the plant was freed of all aphids by means of a pyrethrum-soap spray. This plant continued to grow normally and by early in August had produced a crop of seed without showing any symptoms of disease.

On April 8, 1930, two healthy Erigeron plants in 3-inch pots were set on a 10-inch pot in contact with peach seedlings to which 25 green peach aphids had been transferred from rosette sand cherries on April 2. Aphids readily crawled from the peach seedlings to the Erigeron plants, which were then moved to the greenhouse benches and left for 2 weeks, after which the aphids were killed with a pyrethrum-soap spray. By May 9 these 2 Erigeron plants had reached a height of 12 inches and were apparently quite normal. By August 5 these plants had matured seed without showing any symptoms of disease.

In all cases green peach aphids appeared to thrive on the Erigeron plants after being transferred from the rosette sand cherries.

These tests indicate that the green peach aphid did not transmit rosette from sand cherries to plants of *Erigeron canadensis.*

TRANSMISSION EXPERIMENTS WITH MELON APHIDS

On March 16, 20 melon aphids, *Aphis gossypii* Glover, which had been established for some time on the new growth of rosette sand cherries, were transferred with a camel's-hair brush to a half Petri dish and dropped from there onto 2 healthy Erigeron plants growing in separate pots. The pots were at once enclosed in a bell jar. These aphids failed to become established on Erigeron plants. They crawled about over the plants and pots and onto the soil and the inside of a bell jar but did not appear to feed or reproduce as the green peach aphids had done. Within a few days these aphids all disappeared from the Erigeron plants. The 2 plants continued to grow normally and, by early August, had matured seed without showing any symptoms of disease.

TRANSMISSION EXPERIMENTS WITH BLACK PEACH APHIDS

On April 12, 1930, 22 black peach aphids, *Aphis persicae-niger* Smith, in various stages of development, which had been established on succulent rosette sand-cherry shoots for several weeks, were transferred with a camel's-hair brush to a half Petri dish. From this they were dropped onto 3 healthy Erigeron plants growing in separate pots. After being labeled, the pots were enclosed in bell jars for 4 days; then removed and allowed to grow on the greenhouse bench, after which the aphids were killed with a pyrethrum-soap spray. No symptoms of disease had developed on these plants by early August, when they were maturing seed.

TRANSMISSION EXPERIMENTS WITH MEALY BUGS

Shortly after the potted sand cherries began to develop new rosetted shoots in the greenhouse they became infested with the common mealy bug, *Pseudococcus citri* Risso. These insects appeared to thrive on the rosette sand cherries in the greenhouse but failed to survive when taken to the open.

On March 13, with the aid of a dissecting needle and a camel's-hair brush, 2 of these mealy bugs were transferred from the underside of rosette sand-cherry leaves to a healthy Erigeron plant growing in a 3-inch pot. While the mealy bugs appeared to establish themselves at once on the lower surfaces of Erigeron leaves, they were both found dead when the plant was removed from under a bell jar 5 days later. The Erigeron plant continued to thrive on the greenhouse bench and, by August 12, had matured seed without showing any symptoms of disease.

TRANSMISSION EXPERIMENTS WITH TARNISHED PLANT BUGS

On March 13, 2 tarnished plant bugs, *Lygus pratensis* Linnaeus, captured in a commercial peach orchard, were placed in a porous-cloth bag drawn over a clump of succulent rosette sand cherry shoots. After 3 days in this cloth cage the 2 tarnished plant bugs were freed under a bell jar containing a healthy Erigeron plant. While the bugs were considerably disturbed by their transfer, they soon crawled to the Erigeron plant, where they remained for several days. When the bell jar was removed, later, both bugs were found dead. This Erigeron plant continued normal growth on the greenhouse bench and, by early August, had matured seed without developing any symptoms of disease.

These tests indicate that melon aphids, black peach aphids, mealy bugs, and tarnished plant bugs do not readily transmit rosette from diseased sand cherries to plants of *E. canadensis*.

BUD INOCULATIONS FROM ROSETTE SAND CHERRIES TO PLUMS

To determine the ability of the sand cherry to transmit rosette to other susceptible hosts, as well as to learn whether conditions were favorable for rosette transmission at the time the mechanical and insect transmission experiments were under way in the greenhouse, the following experiment was conducted.

As the rosette sand cherries with which the above greenhouse cross-inoculation experiments were conducted developed as infections from rosette plum buds, it seemed desirable to test the ability of the rosette sand cherry buds to produce rosette in plums. For this purpose dormant wood buds were taken from a branch of 1 of the potted rosette sand cherries April 2, 1930, and put in succulent shoots of plum trees which had been dug from the nursery row, set in 10-inch pots, and brought into the greenhouse December 7, 1929. The buds were tied with ¼-inch rubber strips, but

no attempt was made to force their growth by breaking over or removing the plum tops above the sand-cherry buds. Several weeks later the rubber strips were removed and the buds were found to be united with the stocks. These plum trees continued apparently normal growth in the greenhouse until June 3, when 4 axillary shoots on 1 plum stock below the inserted rosette sand-cherry bud began to show a yellowish appearance of the leaves suspiciously like rosette. No axillary buds had started growth on this stock above the inserted sand-cherry bud, and no axillary bud growth had developed on the other potted plum in which a rosette sand-cherry bud was inserted on the same date.

As the temperature in the greenhouse was becoming too high for good growth, all potted trees except the 2 rosette sand cherries were transferred June 9 to a cold frame near the greenhouse, where they could be watered.

By August 5, 1930, 7 yellowish axillary shoots had developed below the rosette sand-cherry bud, which also had developed a weak rosetted tuft of leaves on the plum that showed 4 axillary shoots by June 3, from rosette sand-cherry bud inserted April 2. In the other plum the sand-cherry bud had developed a short rosetted shoot, but no buds had started on the plum stock. By August 26, when the photograph for figure 3, B, was taken, both of the sand-cherry shoots showed typical rosette, and the plum shoots also showed additional symptoms of rosette in the sprouting of lateral buds on the older axillary shoots below the inserted sand-cherry bud.

Additional symptoms of rosette will develop in the plums inoculated with rosette sand-cherry buds, but the experiment has gone far enough to prove that the potted sand cherries from which the mechanical and insect transfers were made to *Erigeron canadensis* contained the causal agent of rosette. This experiment also proves that conditions in the greenhouse were favorable for the transfer of the causal agent of rosette at the time the cross inoculations were made.

SUMMARY

Hundreds of plants of *Erigeron canadensis* L., raised from seed collected from yellows-infected plants, have shown no symptoms of yellows; therefore, it is concluded that seeds of this wild host do not transmit the causal agent of aster yellows.

Numerous unidentified beetles, and grasshoppers, as well as the tarnished plant bug, *Lygus pratensis* Linnaeus; the potato flea beetle, *Epitrix cucumeris* Paris; the 12-spotted cucumber beetle, *Diabrotica duodecimpunctata* Olivier; the leaf hoppers, *Empoasca mali* LeBaron, *Empoasca flavescens* Fab., and *Cicadula sexnotata* Fall; and the aphids *Rhopalosiphum persicae* Sulzer and *Macrosiphum solanifolii* Ashmead, when transferred with yellows-infected plants of *Erigeron canadensis* and caged on healthy peach trees, failed to transmit any symptoms of disease to the peaches.

More than one hundred mechanical inoculations into peach shoots and green fruits, made with a hypodermic needle and a fresh undiluted extract from yellows-infected plants of *E. canadensis,* gave entirely negative results.

Transmission of rosette from plum to peach and sand cherry by infected buds was 100 per cent effective.

The results of mechanical transmission experiments with the causal agent of rosette from sand cherries to *E. canadensis* were entirely negative.

Experiments on the transmission of the causal agent of rosette from sand cherries to plants of *E. canadensis* by the green peach aphid, *Rhopalosiphum persicae* Sulzer, the black peach aphid, *Aphis persicae-niger* Smith, the melon aphid, *Aphis gossypii* Glover, the common mealy bug, *Pseudococcus citri* Risso, and the tarnished plant bug, *Lygus pratensis* Linnaeus, all proved negative.

Bud transmission of the causal agent of rosette from sand cherries to plums was 100 per cent effective.

The results of these cross-inoculation experiments indicate that aster yellows of the wild host, *E. canadensis,* is a disease distinct from rosette of peach, plum, and sand cherry.

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FURTHER STUDIES ON THE SEED-CORN MAGGOT AND BACTERIA WITH SPECIAL REFERENCE TO POTATO BLACKLEG[1]

J. G. LEACH

In 1925 (5) and 1926 (6) the writer called attention to the rôle played by the seed-corn maggot (*Hylemyia cilicrura* Rond.)[2] in the epiphytology of potato blackleg. Among other things it was pointed out that a number of unidentified species of bacteria were found constantly associated with the insect in all its stages and that some of these were pathogenic and capable of causing blackleg. The bacteria were demonstrated to be present by cultural means only; no histological studies had been made.

It was also shown that sterile maggots would not grow on sterile potato tubers but would grow normally if bacteria were added. This indicated that the bacteria were beneficial to the larvae and under the conditions of the experiment, essential for their normal development.

Since the publication of the above-mentioned papers it has been possible to continue the study of certain phases of the problem. Although the results are by no means complete, what has been learned should be of some aid to a better understanding of the significance of this relationship between insects and bacteria.

THE INTERNAL BACTERIAL FLORA OF THE SEED-CORN MAGGOT COMPARED WITH THAT OF BLACKLEG PLANTS AND WITH CERTAIN SOIL INHABITING BACTERIA

Although pathogenic bacteria were very frequently obtained in culture from the eggs, the larvae, and the pupae, as well as the adult fly, they were never found to occur in pure culture. One or more nonpathogenic species always occurred in association with the pathogenic ones. In some cases only the nonpathogenic species were obtained. Early in the course of the work it was noticed that these nonpathogenic species were apparently similar to the nonpathogenic bacteria frequently found associated with the blackleg pathogene in diseased plants. It was thought desirable to study in some detail these bacteria commonly associated with the insect and to compare them with those associated with the blackleg disease. It

[1] Published with the approval of the Director as Paper No. 964 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Identification of the insects mentioned in this paper, as well as in the previous papers, is based on determinations made by Dr. J. M. Aldrich of the National Museum and Dr. O. A. Johannsen of Cornell University.

also seemed important to isolate and identify the pathogenic species and to determine to what extent they normally were associated with the insect.

Several hundred cultures have been made from internal parts of the larvae, puparia, and adult flies. A considerable number of apparently different species were isolated. Some may have arisen from chance contamination but several were obtained with such frequency that they could be considered as fairly constant inhabitants. Twenty-two of the most frequently occurring species were selected for more careful examination.

Any one who has attempted to obtain a pure culture of the blackleg pathogene from a diseased plant knows that it is sometimes very difficult to separate the pathogene from the nonpathogenic bacteria that are usually closely associated with it. In morphology and cultural characteristics these saprophytes frequently resemble very closely the true pathogene. Five of the cultures most frequently found in association with blackleg were selected for comparison with those obtained from the insect.

In some experiments, in which potato tubers were inoculated with bits of soil or small quantities of soil extract, it was found that a fairly constant group of bacteria were generally associated with the decay that followed. Because the eggs of the seed-corn maggot are commonly deposited in the soil, it was decided to select a number of these soil bacteria for comparison with the other two groups. Several known species of pathogenic, soft-rotting bacteria also were included in the comparison.

At first there was considerable difficulty in obtaining pure cultures of some of the species but, by repeated plating, the different cultures were finally isolated.

These cultures, 43 in all, were studied morphologically and physiologically to determine those characters most commonly used for identifying bacteria.[3]

In these determinations the methods outlined by the Society of American Bacteriologists (1) were followed in general, although in several cases the results were checked by other slightly different methods.

For staining flagella the method described by Gray (2) was used. The method was found relatively simple and quite reliable.

The alkaline gentian-violet Gram stain was employed. Each determination included a gram-positive and a gram-negative organism, one on either end of a slide with the unknown between them.

Pathogenicity was determined by first inoculating carrot slices and potato tuber slices in Petri dishes incubated at 20° C. All cultures that

[3] The major portion of these determinations were made in the laboratory of Professor S. G. Paine, of the Imperial College of Science and Technology. The author wishes to express his gratitude to the Imperial College for the facilities granted and to Professor Paine for helpful suggestions.

produced a decay in these tests were further tested by inoculating potato stems in the greenhouse. Only those that produced a rapid vigorous decay in all 3 tests were considered pathogenic.

The oxygen relations were based on growth in the closed arm of fermentation tubes.

Liquefaction of gelatin was determined by means of a stab culture on plain gelatin, incubating for 2 months at 20° C. followed by a month at room temperature.

The carbohydrate reactions were tested on beef extract to which 1 per cent of the sugar used was added before sterilization.

Litmus indicator and Dunham tubes were used for the detection of acid and gas.

The reduction of nitrates was detected by the sulphanilic acid-α-naphthylamine test.

Diastatic action was determined on 2 per cent soluble-starch beef-extract agar tested with a saturated solution of iodine in 50 per cent alcohol.

The Ehrlich test for Indol was employed, and in all cases where positive results were obtained the reaction was very marked.

The reaction of all differential media was adjusted to the neutral point of brom-thymol-blue.

No determination was based on a single test. Duplicates or triplicates were always used and doubtful reactions were always repeated until conclusive results were obtained.

The principal characters as determined are summarized in table 1. In analyzing the data in this table it should be realized that no atttempt was made to study all the different kinds of bacteria found associated with the insects or the disease. The coccus forms and large, spore-forming bacteria were excluded, although they may possibly be of some significance. Many of the cultures were isolated while searching for the pathogene and this without doubt resulted in a certain amount of selection of those species resembling the blackleg pathogene.

The important facts demonstrated by the detailed study of this group of cultures may be summarized briefly as follows:

1. Bacteria, apparently identical morphologically, physiologically, and parasitically with known cultures of the blackleg pathogene, were frequently isolated from the surface of the eggs, from the inside of puparia, and from the intestinal tract of the imago of the seed-corn maggot, as well as from the soil and from potato plants affected with blackleg. (Cultures No. 151–A–B, 201–2–R, 202–B–I–R, 301–1–R, 305–A–O–R, 4–I, and 407.)

2. Bacteria, which agreed morphologically and physiologically with published descriptions of *Pseudomonas fluorescense* (Flügge) Migula, and

TABLE 1.—*Summary of characteristics of 36 cultures of bacteria obtained from the seed-corn maggot in various stages of development, from blackleg plants, and from the soil. Seven cultures of soft-rotting bacteria of known identity are included for comparison*

Culture No.	Source	Index number and brief characterization[a]			
151–A–O	Surface of egg of seed-corn maggot	5020–52020–0211–2210–211	22	UUU	0
151–A–R	"	5010–32120–0111–2210–211	22	"	0
153–B	"	5010–32020–0111–2210–211	22	"	0
154–B	"	5020–52101–0200–2210–211	22	"	0
155–A–O	"	5020–52001–0200–2210–211	22	"	0
201–A–R–G	Inside of puparia of seed-corn maggot	5020–52020–0101–2210–211	22	"	0
201–A–R–W	"	5020–52020–0101–2210–211	22	"	0
201–A–2	"	5020–52100–0000–2210–111	22	"	0
201–2–R	"	5010–32120–0111–2210–111	22	"	0
201–A–3	"	5020–52001–0200–2210–211	22	"	0
201–B–1	"	5020–52001–0200–2210–211	22	"	0
201–B–R	"	5020–32120–1111–2210–111	22	"	0
202–B–1	Intestinal tract of imago of seed-corn maggot	5010–52000–0000–2210–211	22	"	0
202–B–I–R	"	5010–32120–0111–2210–211	22	"	0
202–B–O	"	5020–52100–0000–2210–111	22	"	0
203–A	"	5020–52020–0100–2210–211	22	"	0
204–A–G	"	5010–52100–0222–2210–111	22	"	0
204–A–W	"	5010–52100–0202–2210–111	22	"	0
205–A–1	"	5020–52101–0200–2210–211	22	"	0
205–A–O	"	5010–52020–0100–2210–211	22	"	0
205–A–R–G	"	5020–52020–0100–2210–211	22	"	0
205–A–R–W	"	5020–52001–0200–2210–211	22	"	0
301–1–R	Soil	5010–32120–0111–2210–211	22	"	0
301–2	"	5010–52020–0000–2210–111	22	"	0
302–1	"	5010–52120–0000–2210–111	22	"	0
302–2	"	5010–52020–0000–2210–211	22	"	0
303–1	"	5020–52100–0000–2210–111	22	"	0
303–2–R	"	5010–52120–1111–2210–111	22	"	0
305–A–1	"	5020–52001–0200–2210–111	22	"	0
305–A–O–R	"	5010–32120–0111–2210–211	22	"	0
306–R	"	5020–32120–1111–2210–111	22	"	1
404–4	Blackleg plants	5010–52120–0101–2210–211	22	"	0
407	"	5010–32120–0111–2210–211	22	"	0
408–A–1	"	5020–52101–0200–2210–211	22	"	0
409–2–R	"	5020–52001–0200–2210–211	22	"	0
4–I	"	5010–32120–0111–2210–211	22	"	0
Bacillus phytophthorus Appel I	Furnished by E. F. Smith	5010–32120–0111–2210–211	22	"	0
B. carotovorus	Furnished by A. B. Massey	5010–32120–0111–2210–211	22	"	0
B. phytophthorus 1985L	Furnished by Lister Institute	5010–32120–0111–2210–211	22	"	0
B. phytophthorus 1975L	"	5010–32120–0111–2210–211	22	"	0
B. phytophthorus 1996L	"	5010–32120–0111–2210–211	22	"	0
B. solanisaprus 385L	"	5010–32020–0111–2210–211	22	"	0
B. aeroideae 1984L	"	5010–32120–0222–2210–211	22	"	0

[a] Numbers correspond to those used in the description chart of the Society of American Bacteriologists.

Ps. non-liquefaciens Eisenberg, were isolated from the surface of eggs, the inside of puparia, and the intestinal tract of the imago of the seed-corn maggot, as well as from the soil, and plants affected with blackleg. (Cultures No. 154–B, 155–A–0, 201–A–3, 201–B–1, 205–A–1, 205–A–R–W, 305–A–1, 408–A–1, 409–2–R.)

3. Several other nonpathogenic species of bacteria were found to occur commonly in association with the seed-corn maggot, in the soil, and in plants affected with blackleg.

4. No consistent morphologic, physiologic, or parasitic difference could be found between any of the cultures of the blackleg pathogene with the exception that the culture of *Bacillus solanisaprus* Harrison did not liquefy gelatin, while all other pathogenic cultures liquefied it readily and consistently.

5. *Bacillus aeroideae* Townsend, although differing from other pathogenic species in its action on the sugars, was strongly pathogenic on potato tubers and stems.

The similarity of the bacteria making up the intestinal flora of the seed-corn maggot to those obtained from the soil and from plants affected with blackleg appears to be significant. It is probable that the kinds of bacteria found in the intestines of the insect depend largely upon the substrate upon which the insect has fed. An insect having developed in a plant affected with blackleg would probably harbor the blackleg pathogene as well as some of the associated saprophytes, while one having developed in decaying organic matter in the soil probably would harbor only those saprophytes commonly predominating in the soil or in decaying organic matter.

HISTOLOGICAL STUDIES

Although bacteria may be isolated from the interior of maggots, puparia, and adult insect, their exact location within the body can be determined only by histological methods. Furthermore, since it has not been possible to propagate the insect in cages, histological studies seem to offer the best means of determining whether the bacteria are of any significance in the metabolism of the adult fly. Maggots, puparia, and adults of both sexes have been studied histologically. The results of the study to date have been unsatisfactory in some respects but some significant observations have been made. These will be recorded in this paper, and it is hoped that future studies will clear up some of the yet obscure points.

Maggots and puparia in various stages of development were collected in the field during May and June. Some adults were caught in the field, while others were reared from maggots in the laboratory. All specimens used for sectioning were killed and fixed in Zenker's Solution and embedded in paraffin. Sections were cut, varying from 5 to 12 microns in thickness.

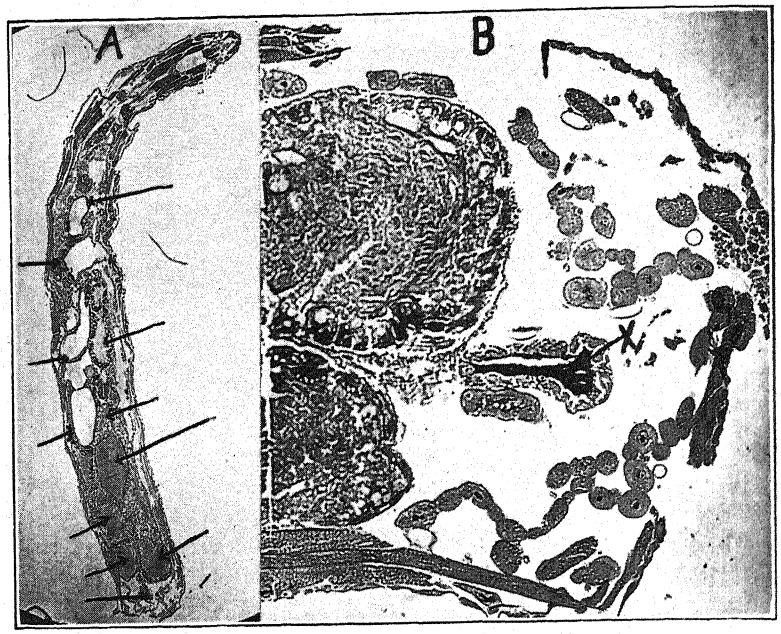


FIG. 1. Photomicrographs showing sections through a larva. A. A longitudinal section; the arrows show the location of intestinal tract containing bacteria mixed indiscriminately with food material. See figure 3 for magnification sufficiently high to show individual bacteria. The bacteria were not concentrated in any special organs. There was no indication that the bacteria were killed or digested by the larva. Approx. 12 ×. B. Enlarged section of posterior end showing bacteria in anal tract at X. Approx. 100 ×.

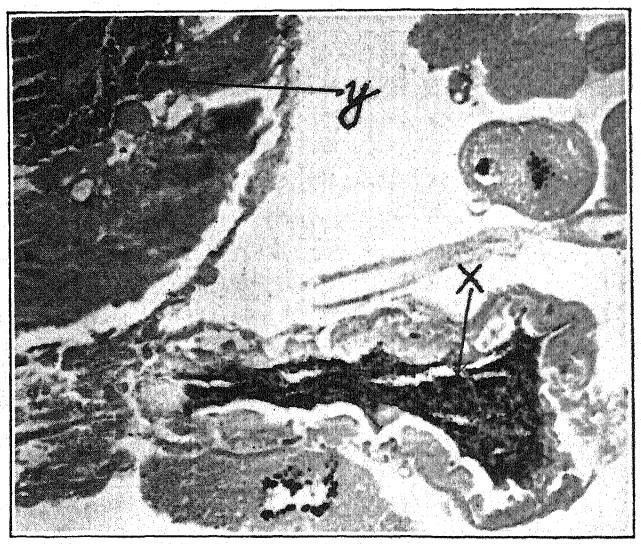


FIG. 2. Same as Fig. 1, B, more highly magnified, showing bacteria at X and Y. Approx. 260 ×.

Two staining methods were used: that of Goodpasture, as described by Hertig and Wolbach (3); and the Gram-Weigert method, described by Wright and Mallory (8). Aid in interpreting stained microtome sections was obtained by dissecting a large number of freshly killed flies and maggots.

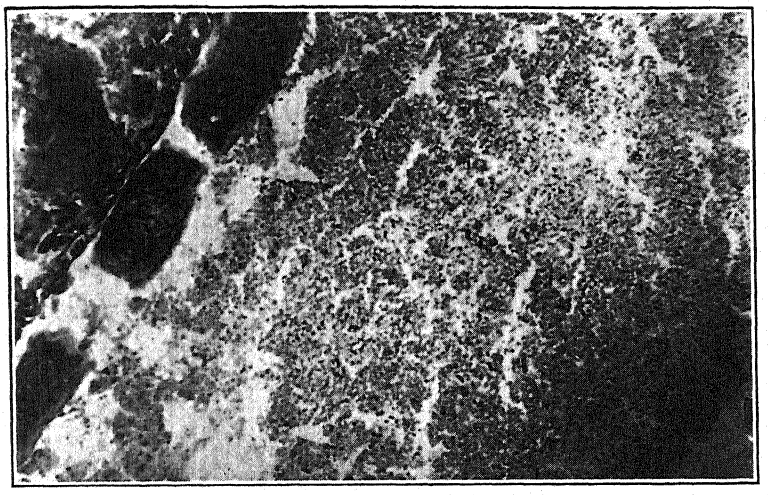


FIG. 3. Section of intestinal contents of a larva, showing short rod-shape bacteria mixed with food material. Approx. 300×. This is from the same larva illustrated in figure 1, A.

The Larva

Although there was a tendency for the larvae to become abnormally lengthened and somewhat discolored during the fixing process, the material yielded fairly good sections which stained well with the Gram-Weigert stain. Bacteria in great abundance were found in the intestinal tract (Figs. 1, 2, and 3). They were in a mixed culture, as indicated by the wide variation in size and shape of the individual cells (Fig. 3). No selective action on the part of the maggot could be detected. The bacteria were mixed indiscriminately with the food material and were not localized in the coecal glands or other special organs. Four long finger-like coecal pouches were found arising immediately behind the proventriculus (Fig. 4), but these did not contain bacteria. Numerous smear stains from freshly dissected material, as well as stained microtome sections, showed them filled with a mixture of spherical and granular bodies embedded in a clear liquid (Fig. 5). However, bacteria were found in the cavity of the proventriculus surrounding the oesophageal valve (Figs. 4 and 6). This cavity may correspond to the "blind sacks" containing bacteria described by Stammer (11), but, if so, it is greatly reduced.

There was no evidence that the bacteria were digested or destroyed by the larva, and they could be found in almost an unbroken mass from one end of the intestinal tract to the other (Figs. 1 and 2). It is quite evident that living bacteria of many different species pass uninjured through the intestinal tracts of the larvae.

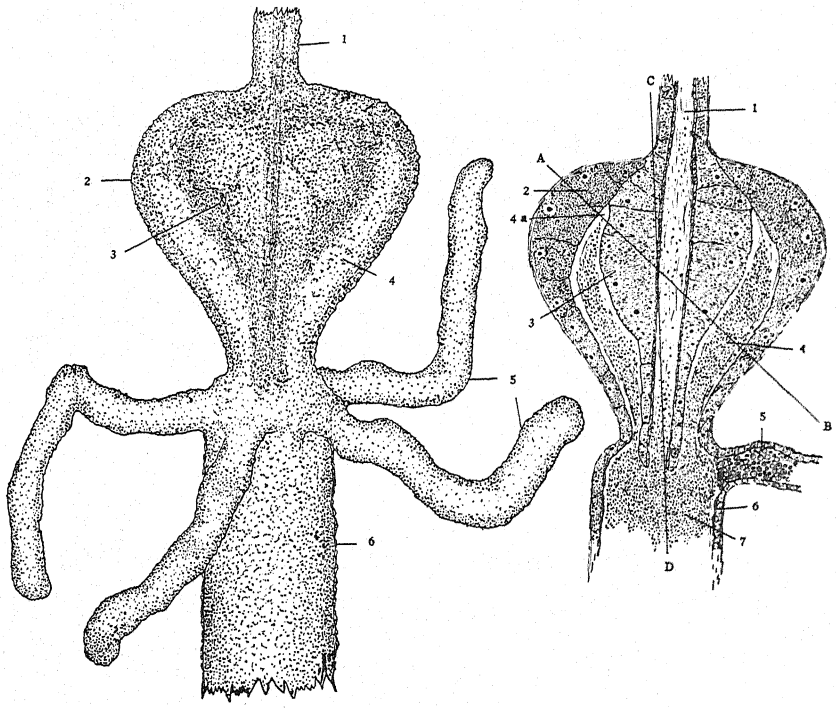


FIG. 4. Semidiagrammatic drawings to show the distribution of bacteria in the proventriculus and anterior portion of the midintestine of a seed-corn-maggot larva. A surface view as seen when dissected under a binocular microscope (at left) and as seen in longitudinal section (at right). 1. Oesophagus. 2. Enlarged portion of midintestine surrounding the oesophageal valve and commonly termed the proventriculus. 3. Oesophageal valve dimly outlined through the wall of the midintestine. 4 and 4a. A space surrounding oesophageal valve containing bacteria and food material. 5. The four coeca filled with spherical bodies of irregular size but containing no bacteria. 6. Midintestine. 7. Bacteria and food materials. For explanation of diagonal lines see figure 6.

The Puparia

The histological studies of the puparia were very unsatisfactory. It was necessary to puncture the puparial case to insure proper fixing; and in certain stages of metamorphosis this apparently resulted in decided disorganization of the enclosed tissues. Sometimes fixation was apparently not complete. In other cases fairly good sections were obtained but the tissues

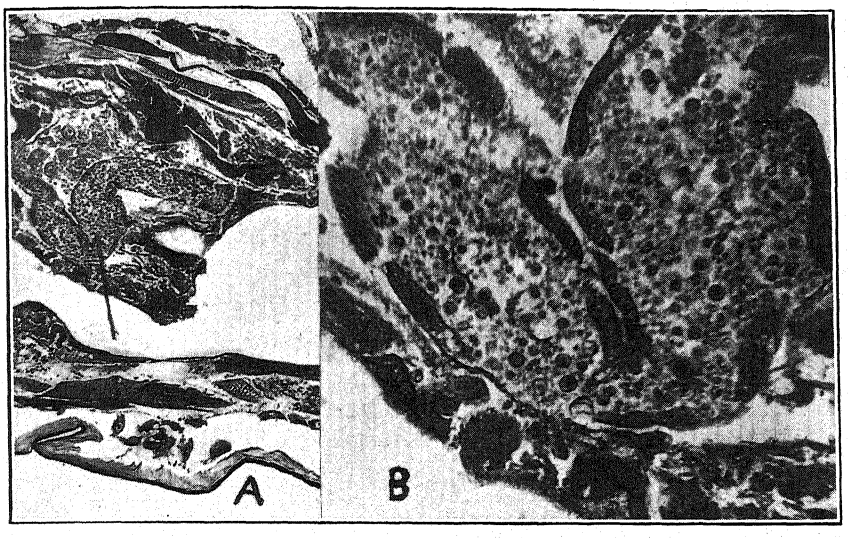


FIG. 5. A photomicrograph of sections through a coecum showing the spherical bodies filling the lumen. A. Magnified approximately 65×. Coecum indicated by arrow. B. Same (approximately 325×), showing the spherical bodies and the absence of bacteria.

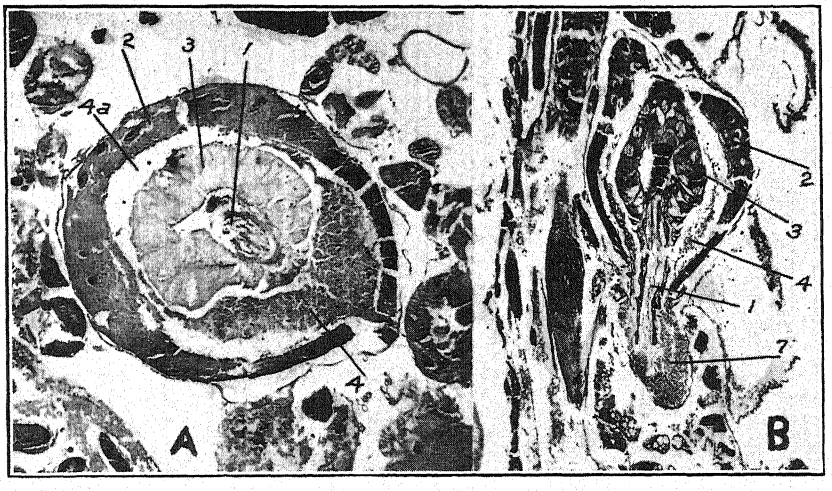


FIG. 6. Photomicrographs of sections through the proventriculus, showing bacterial masses surrounding oesophageal valve. A. Cut in plane indicated by line AB. in figure 4. B. Cut in plane indicated by line CD. in figure 4. Numbers correspond to those in figure 4.

did not stain well. In all, approximately 50 different puparia were sectioned and stained. Nearly every possible stage in metamorphosis was included among these but in none of them was it possible to locate bacteria with any degree of certainty. The bacteria apparently lose their affinity for the stain or else become greatly reduced in number. Although the presence of bacteria within the puparia can be demonstrated easily by cultural methods, it has not yet been determined which tissues they inhabit during the metamorphosis of the insect. The solution of this problem must await further work.

The Imago

In order to facilitate fixing the adult insect, the abdomen was usually severed from the thorax. The fixing was satisfactory and the section stained fairly well by both of the staining methods used. Bacteria were rarely found in the thoracic segment but were nearly always present in the abdominal segment in the crop and alimentary canal (Figs. 7 and 8). The number of bacteria present varied with different specimens, apparently depending upon the age of the insect and the amount of food in the intes-

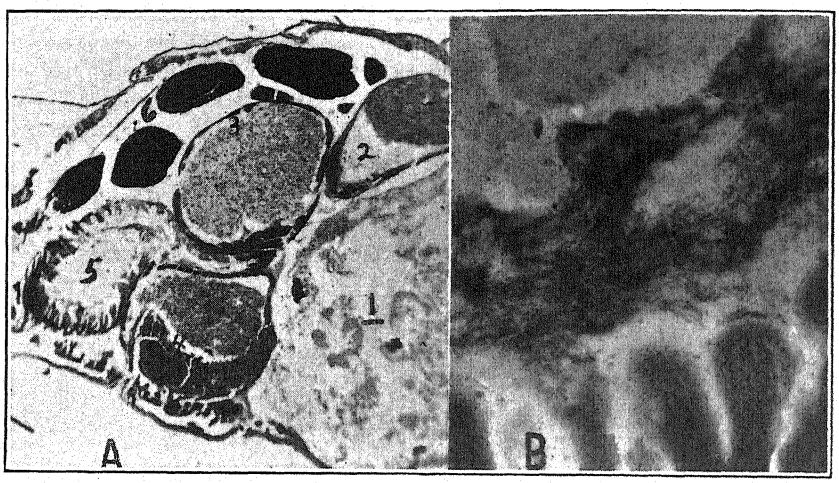


FIG. 7. Photomicrographs showing bacteria in crop and intestinal tract of the imago. A. Dorsoventral longitudinal section of the abdomen of a female imago. 1. The crop containing a liquid matrix in which quantities of bacterial cells are suspended. Higher magnifications are shown in figure 5. 2, 3, and 4. Cross sections of the intestine containing semi-solid mass composed largely of bacterial cells of various sizes and shapes intermixed with food materials. 5. A more posterior section of the midintestine in which a mass of uniform rod-shope bacteria are found in a compact layer surrounding a homogeneous mass of food materials. 6. Immature eggs (approx. 25 ×). B. A more highly magnified view of the bacterial mass in the midintestine shown in A, 5, and in figure 8. (Approx. 400 ×.)

tinal tract. If the intestinal tract contained a large quantity of food material, the bacteria were usually numerous; if the intestines were virtually empty, the bacterial content was also low. In general, food materials and bacteria were more abundant in the females than in the males.



FIG. 8. Photomicrograph showing bacteria surrounding the homogeneous mass of food materials in the midintestine. A higher magnification of figure 7, A, 5 (approx. 200 ×).

The crop and the anterior portion of the midintestine usually contained a mixed culture of bacteria of all sizes and shapes mixed uniformly throughout the food material, presenting a picture very similar to that found in the intestines of the larva (Fig. 9). In many specimens there was some evidence of destruction and disappearance of the bacteria as they passed along the intestinal tract. The destruction, however, was incomplete and appeared to be somewhat selective in its action. By the time the food had reached the posterior portion of the midintestine all the bacteria had disappeared except a group of short rod-shape bacteria that had accumulated in a layer surrounding the homogeneous food material and lying between it and the walls of the intestine (Figs. 7, 8, 10, and 11). The walls of the midintestine are at this point lined with papillose epithelial cells. A careful examination under high magnification showed the bacteria in the space between the food contents of the intestine and a thin transparent peritrophic membrane. They were all short rods and were very uniform in size and shape. The regular arrangement of the cells in definite chains would appear to indicate that these bacteria were in a congenial

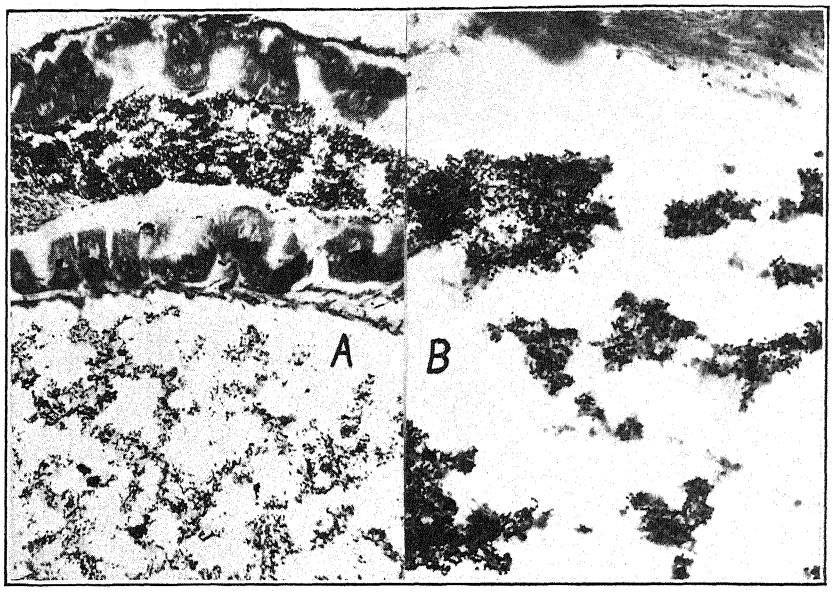


FIG. 9. Photomicrographs showing bacteria in crop and midintestine of the imago. A. Section showing bacteria in intestinal tract (at the top) and in the crop (at the bottom). (Approx. 100 ×.) B. Higher magnification of bacteria in the crop. (Approx. 350 ×.)



FIG. 10. Photomicrograph showing a section of the hind intestine with chains of rod-shape bacteria surrounding the granular residue of food materials. (Approx. 250 ×.)



FIG. 11. Photomicrograph showing a mass of short, rod-shape bacteria of uniform size between the granular residue of the food materials and the walls of the hind intestine. (Approx. 500 ×.)

environment and were actively growing and multiplying. From this point the food materials rapidly disappeared, but the short rod-shape bacteria could be traced throughout the length of the intestine to the anal opening (Figs. 12 and 13). These bacteria, as previously demonstrated culturally (6), pass out in the feces in a viable condition. In certain specimens very

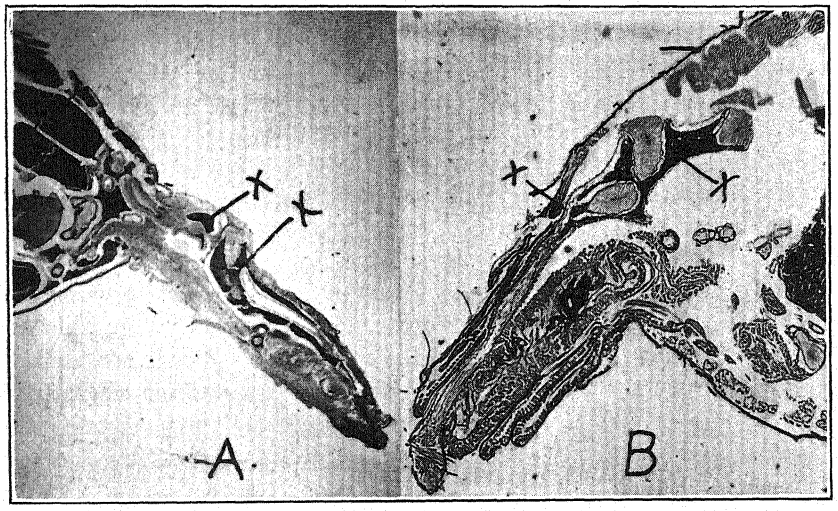


FIG. 12. Photomicrographs showing sections through the rectum and posterior end of the intestinal tract showing, at X, masses of apparently uninjured bacteria. A. Approx. 20 ×; B. Approx. 30 ×.

little food material was found in the intestines. In such specimens small numbers of the bacteria were nearly always found adhering in groups or chains to the peritrophic membrane (Fig. 14).

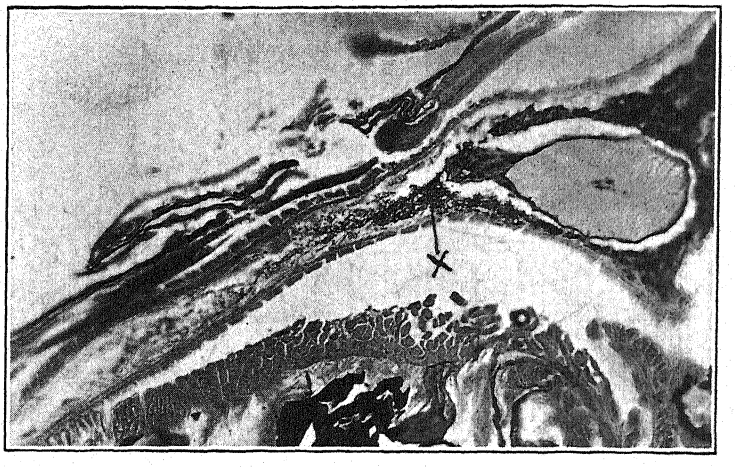


FIG. 13. Photomicrograph showing bacterial masses in the rectum and in passage leading to anal opening. The same section shown in figure 9, B. These bacteria are all short rods measuring about 0.5 microns x 1½ microns and are all apparently uninjured. They obviously are excreted in a viable condition. (Approx. 100 ×.)

It is of interest to compare the histological relationship between bacteria and the seed-corn maggot with that found in related insects by other workers. Petri (10) in 1910 published an account of his studies of the olive fly (*Dacus oleae*). He showed that a symbiotic relationship existed between this insect and several species of bacteria among which *Bacterium savastanoi* E. F. S. (the olive-knot pathogene) was almost universally present. He found the bacteria constantly localized in 4 spherical blind sacks arising from the forepart of the midintestine of the larva. They also were found at times in the lumen of the intestines and were frequently passed out with the excrement.

Petri showed that the bacteria survived during pupation and were present in the body of the imago upon emerging from the puparium. Shortly before pupation most of the bacteria were expelled by the larva but a few migrated to the forepart of the oesophagus. They were harbored here until a special sack-like outgrowth appeared above the pharynx, in which they developed in large numbers. After emergence of the imago the bacteria spread throughout the intestinal tract. In female flies they soon became located in a series of small glandular sacks near the anal opening. These glandular sacks were so situated that the eggs, on passing out of the ovipositor, became surface-contaminated with the bacteria. The

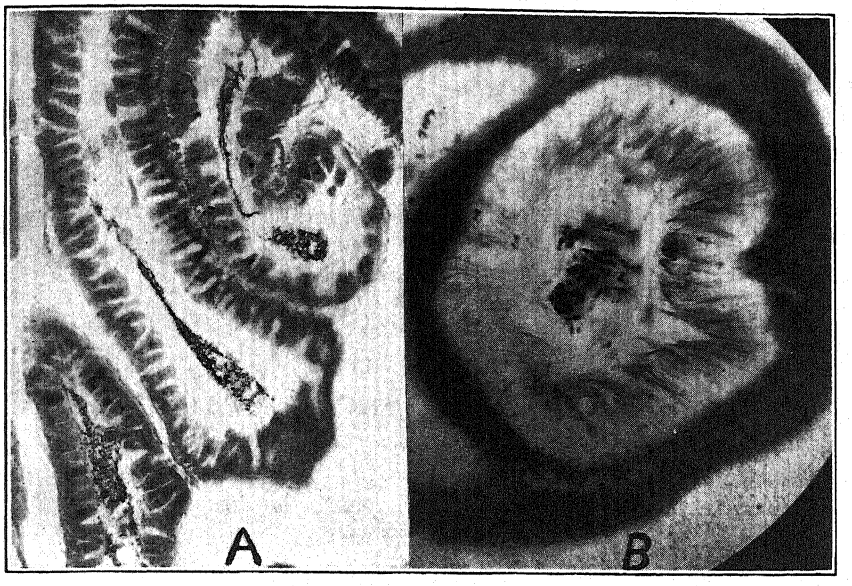


FIG. 14. Photomicrograph showing sections of mid- and hind-intestines containing very little food material but with masses of bacteria adhering to the peritrophic membrane. A. Midintestine. (Approx. 75 ×.) B. Hind-intestine near rectal valve. (Approx. 600 ×.)

bacteria multiplied rapidly in the gelatinous coating over the egg and migrated through the air canals around the micropyle, thus infecting the embryo. In this way the continued association of the bacteria and insect was insured.

Stammer (11) has recently confirmed the histological findings of Petri and has also investigated histologically numerous other representatives of the Trypetidae. He found symbiotic bacteria present in all of the representatives of this group that he studied. In some species the bacteria were harbored in special blind sacks off the intestines of the larvae, while in other species they occurred free in the intestinal tract. In some species special devices for insuring the contamination of the eggs were found in the adult female; in others no such structures were present. Where these structures occurred they were much simpler than those found in *Dacus oleae* consisting principally in the union of the anal tract and the vagina into one common opening.

In the imago of the seed-corn maggot no such structures were found, and, although the openings of the anus and vagina are very close together, no direct union was observed. Also, as pointed out above, the bacteria were not concentrated in special organs but were found throughout the intestinal

tract. The symbiotic relationship here, therefore, appears to be of a very simple type. This is probably what would be expected when the life habits of the insect are considered. Here the larvae spends its life in a mass of decaying plant tissue, surrounded by a constant supply of bacteria. The presence of such special organs for harboring the bacteria would appear superfluous. Likewise, the eggs being deposited in the soil, the larvae have abundant opportunity to become contaminated with the bacteria commonly associated with it. A special structure to insure contamination would appear of less significance in the seed-corn maggot than in those insects that deposit their eggs directly into plant tissues.

NUTRITIONAL STUDIES

Preliminary experiments reported in 1926 (6) showed that sterile larvae were unable to grow when placed on sterile nutrient agar or sterile potato plugs. If, however, bacteria were added to the medium, they would grow and pupate normally. Although these experiments demonstrated that the bacteria played an important rôle in the metabolism of the insect, no attempt was made to explain the exact relationship. Further studies have thrown some additional light on the problem. Since the publication of the paper mentioned above (6), Huff (4) has reported the results of his nutritional studies on the seed-corn maggot. He also found that sterile larvae would not grow to maturity on sterile beef-extract agar or potato plugs but that they would grow normally on contaminated agar or potato plugs.

Huff was unable to grow the larvae on bacteria-free filtrate from unheated potatoes or on sterile potato to which had been added a suspension of bacteria killed by heat. On the other hand, the larvae grew normally on potato plugs, beans, and peas that had been partially decomposed by bacteria and then sterilized by heat. He was able to grow the larvae to maturity on sterile germinating seedlings also. From these results he concluded that the bacteria, *per se*, are not essential to the development and pupation of the larvae but that, by their digestive action, they convert the plant tissue into available food for the larvae. The presence of available food in the sterile germinating seedlings is assumed to be due to the natural processes of digestion which occur when seeds germinate. Similar experiments have been made by the writer and the results obtained agree in all essential details with those of Huff and tend to bear out his general conclusions. One point of difference, however, should be mentioned. Huff states that the larvae grew rapidly and pupated in the normal length of time while feeding on sterile seedlings. In the writer's experiments, although larvae grew to maturity on sterile seedlings, they always grew more slowly than larvae on contaminated seedlings and a much lower percentage pupated normally.

DISCUSSION AND CONCLUSIONS

From the evidence presented here and in previous publications, it is possible to draw some general conclusions concerning the nature of the relationship between the seed-corn maggot, the blackleg disease of potatoes, and the associated bacteria. The evidence shows that the seed-corn maggot is a common pest of the potato plant. The adult female fly deposits its eggs on the seed piece or in the soil near the base of the plant. When the eggs hatch, the larvae attack the seed piece and at the same time inoculate it with a mixed culture of bacteria that cause a certain amount of decay. In some cases the bacteria may consist of only the saprophytic species that make up the normal soil flora and that find the potato tubers a favorable substrate for growth. These saprophytes are able to rot the tubers only when aided by the destructive action of the larvae. In other cases the blackleg pathogene may be included among the bacteria making up the inoculum. As pointed out previously by the writer (6), the blackleg pathogene can be introduced on the eggs of the insect or in the feces of the imago. Since it also has been demonstrated by the writer (7) and Patel (9) that the blackleg pathogene can survive in the soil, it is reasonable to assume that it can be inoculated into the potato seed piece by the larvae along with other bacteria from the soil. When the blackleg pathogene is introduced, other conditions being favorable, the plant will succumb to the disease.

As shown by the nutritional experiments of Huff (4) and the writer, the bacteria aid the larvae in their development by digesting the tissues of the potato, thus making them more available as food. In all probability the bacteria as such are not utilized as food by the larvae. This view is supported by the histological studies which show that the bacteria are not digested or destroyed but are passed through the intenstinal tract without any apparent injury.

When the larvae pupate some of the bacteria among which they are feeding apparently are retained within the puparial case and are to be found in and on the body of the imago as it emerges. This has been demonstrated repeatedly by cultural methods, although the histological details have not been satisfactorily determined. If the larva has fed in a potato plant affected with blackleg, it is only reasonable to expect the blackleg pathogene to be present in the puparium and in the imago. This has been shown true by numerous cultural and inoculation experiments (6). It has been demonstrated also that the adult flies feed upon liquids containing bacteria and that bacteria, including the blackleg pathogene, are frequently found in the intestinal tract and can be passed out in a viable condition in the feces.

For practical considerations it should be recognized that the dissemination of the pathogene is incidental from the standpoint of the insect.[4] Although some kinds of bacteria are always associated with the insect in various stages of development, the presence of the blackleg pathogene is not essential. However, if the insect has fed on tissues affected with the disease, the pathogene is likely to be present. It should not be assumed that blackleg will necessarily develop in every plant attacked by the insect. If the pathogne is not introduced with the eggs and if the larvae do not introduce it from the soil there is no reason to expect the disease to develop. When the pathogene is present, on the other hand, the larvae serve as effiicient agents of inoculation and aid infection and development of the disease.

When these facts are considered, it is obvious that the origin of flies which deposit their eggs upon a potato plant may determine the extent to which they disseminate the pathogene. Flies arising from larvae that have developed in blackleg plants are more likely to have the blackleg pathogene in their system than those from larvae developed in other decaying organic matter. Numerous isolations from flies from various sources have tended to verify this assumption. There is also some evidence to show that this insect is a more frequent carrier of the disease in regions where potatoes are intensively grown.

SUMMARY

1. A comparative study has been made of the internal bacterial flora of the seed-corn maggot, the principal bacteria associated with the blackleg disease of potatoes, and certain soil-inhabiting bacteria. Several species occurring in each of these groups were found identical. The blackleg pathogene was obtained from each of these three groups, although certain soil saprophytes, including *Pseudomonas fluorescense* (Flügge) Migula and *Ps. non-liquefaciens* Eisenberg, appeared to predominate.

2. It is concluded that the kinds of bacteria associated with the insect are determined largely by the nature of the material on which it feeds. Flies that have developed in potato plants affected with blackleg would be more likely the carriers of the pathogene than those developing in organic matter destroyed by the common soil saprophytes.

3. Histological studies of the relationship between the seed-corn maggot and bacteria are reported. These studies indicate that bacteria of many species pass uninjured through the intestinal tract of the larvae. The

[4] In an abstract of this paper (Phytopath. 20: 127, 1930) the insect was termed an "occasional" carrier. "Incidental" is preferable, inasmuch as occasional often conveys the idea of infrequency, while incidental does not involve the question of frequency. The dissemination of the blackleg pathogene by the seed-corn maggot is always incidental, although it occurs frequently.

larvae apparently do not feed upon the bacteria as such. Efforts to follow the course of the bacteria in the puparium during metamorphosis were unsuccessful. In the imago, also, bacteria were found to pass through the intenstinal tract without injury. Here it appeared that certain species of bacteria were destroyed and digested by the insect, while certain short rod-shape species were uninjured and apparently grew and multiplied in the posterior portion of the tract.

4. In neither larva nor imago were the bacteria found to be harbored in special organs but were constantly present in the lumen of the intestines. In the larva bacteria were present in the proventriculus or enlarged portion of the midintestine surrounding the oesophageal valve. In the imago the bacteria were present in the crop and intestinal tract. In the crop and a portion of the midintestine the bacteria were scattered throughout the food material, but in the more posterior portions of the intestinal tract they were accumulated in a layer between the food mass and the peritrophic membrane. Their arrangement in definite chains indicated that growth and multiplication had occurred in this location.

5. No special devices for insuring contamination of the eggs were observed. The histological relationship between bacteria and this insect are considered to be of a simpler type than most of the Trypetidae studied and described by Stammer (11).

6. Nutritional studies with the larva of the seed-corn maggot indicate that bacteria aid its development by transforming the plant tissues into a form more readily assimilated. The ability of the larva to grow normally on seed partly decayed by bacteria and then sterilized by heat, as well as on sterile germinating bean seed, indicates that the bacteria are not utilized as food by the insect but that they digest the plant tissues and make them available as food for the larva.

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THE LIFE HISTORY OF SCLEROTINIA SCLEROTIORUM WITH REFERENCE TO THE GREEN ROT OF APRICOTS

RALPH E. SMITH

One of the most characteristic fungus diseases of central California is the so-called green rot of apricots. It is characteristic in its sporadic occurrence, breaking out locally in virulent epiphytotic form and causing almost complete crop losses in certain seasons, then disappearing entirely for a period of several years. The first recorded instance of this kind was in 1910 when the apricot crop in the district about the southern end of San Francisco Bay was greatly reduced by this disease. In 1916 and 1928 further epiphytotics occurred. Since then no outbreak of green rot has taken place in that district. Since 1910 there has been a considerable planting of apricots in the San Joaquin Valley, mainly in Stanislaus and Merced counties, of the late, slow-blooming Tilton variety. In 1928 and several preceding years losses from green rot were severe in this district.

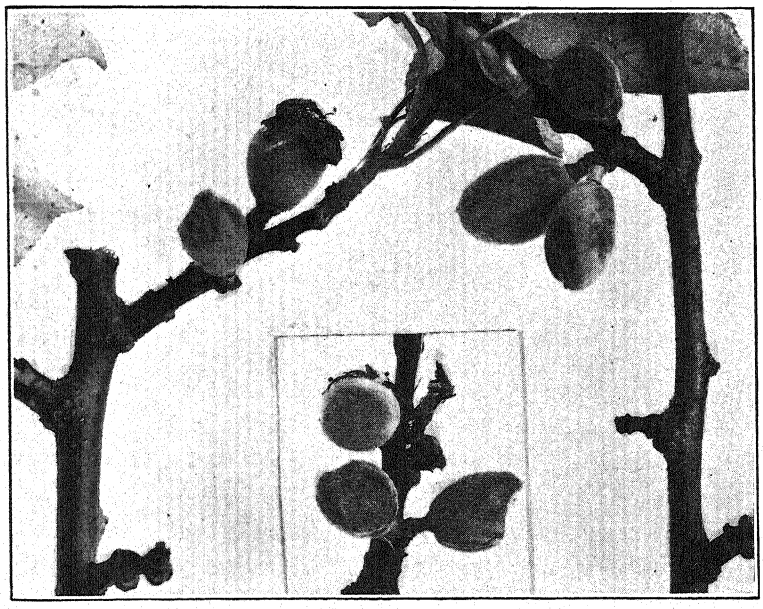


FIG. 1. Green rot of apricot. Infection of young fruit through dried calyx by *Sclerotinia sclerotiorum*.

The disease consists in a complete or partial destruction of the young fruit, commencing while it is still in the "jacket" stage (enclosed in the

dry calyx (Fig. 1). It is entirely distinct from the blossom-blight form of "brown rot" which is caused by *Sclerotinia cinerea* (Bon.) Schr. in that it attacks the young fruit through the dead calyces rather than the living petals of the blossom (Fig. 2). The tree is not ordinarily attacked by this disease in any part except the blossom and the young fruit. In 1928 almonds were quite generally attacked in the same manner as the apricot.

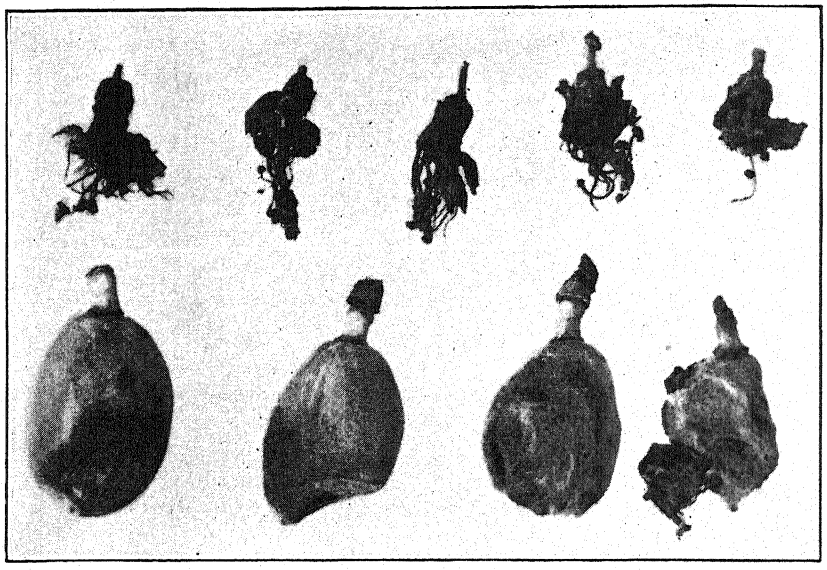


FIG. 2. Young apricots (below) attacked by *Sclerotinia sclerotiorum* through dead calyx, contrasted with blossom-blight effect of *S. cinerea* (above).

The idea is generally accepted that the cause of this disease is the common cottony mold fungus, *Sclerotinia sclerotiorum* (Lib.) Mass. A fungus of this type may be regularly and easily cultured from affected fruit. The typical mycelium and sclerotia develop profusely from such material either in a moist chamber (Fig. 3, A) or, in wet weather, on moist soil in the orchard. The fungus readily attacks the young fruit in the typical manner when inoculations are made either with mycelium or ascospores. Typical apothecia develop from the sclerotia (Fig. 3, B). This has been observed in the soil under apricot trees on sclerotia kept in moist sand and even in flask cultures (Fig. 3, C).

Sclerotia have never been observed upon the tree; in fact, the fungus makes almost no visible development at the points of attack. The young fruits are killed and drop to the ground or may even resist the attack sufficiently to develop to maturity marked with a scar or lesion, as in figure 2.

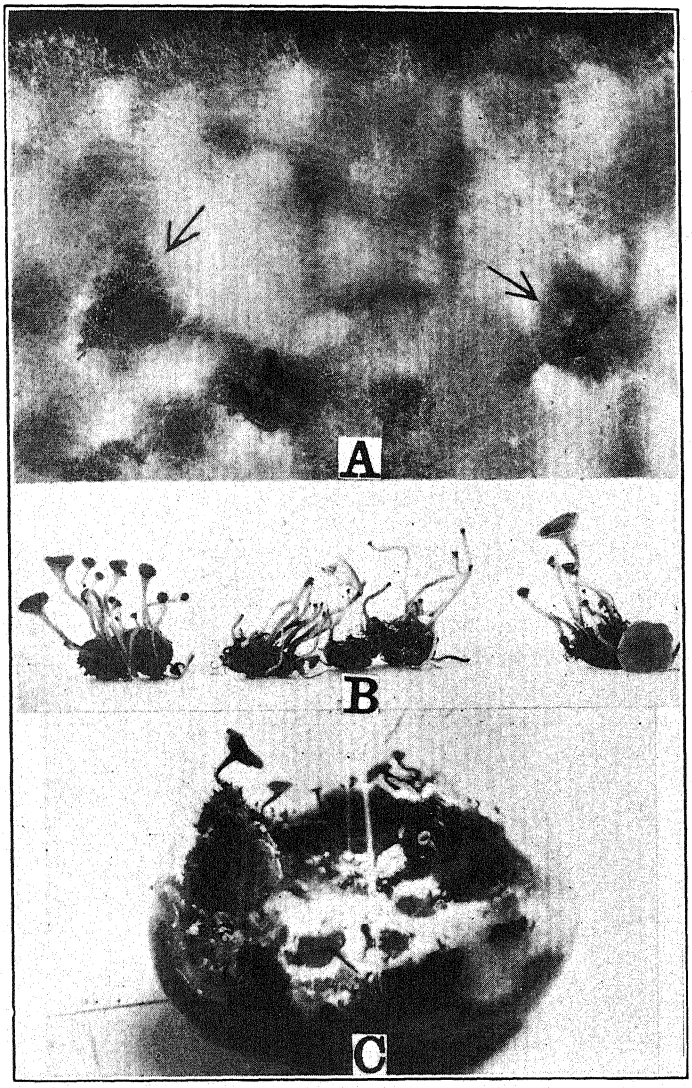


FIG. 3. A. Mycelium of *Sclerotinia sclerotiorum* from young apricots and blossoms after 2 days in moist chamber. Conidiophores of *Botrytis cinerea* also present at arrows. B. Sclerotia and apothecia of *S. sclerotiorum* from soil in apricot orchard. 1916. C. *S. sclerotiorum* producing apothecia from sclerotia in flask culture. 1916.

Any further development of the fungus and, particularly, so far as known, the formation of any spore stage take place only in the soil. The apricot and more rarely the almond (Fig. 4) are the only known hosts of this type of disease. Although, as shown in table 1, spores of the fungus are abundant on flowers of the cherry, prune, and peach, and many annual plants, no natural infection of these hosts in the manner of aerial attacks of the blossoms has ever been observed.

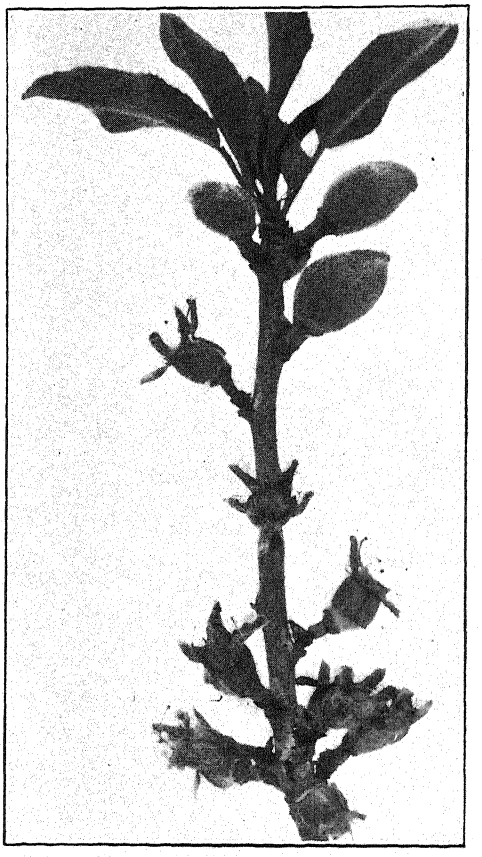


FIG. 4. Young fruits of almond in jacket, when infection with *Sclerotinia sclerotiorum* takes place.

Nixon and Curry (2) state that they produced typical green rot of apricots by inoculation with spores of *Botrytis cinerea* obtained by culturing the diseased fruit and think that the disease may be caused by various fungi, including Botrytis and Sclerotinia. Smith and Smith (5, p. 1097) expressed the same idea, stating that green rot is "caused by vari-

ous fungi of which perhaps a species of Sclerotinia, apparently *S. libertiana,* is most common, causing a decay of the young fruit on the tree. When such fruit is picked and placed in a moist chamber it develops an abundant cottony mold in which black sclerotia soon form. . . . *Botrytis vulgaris* is also common in this trouble."

C. O. Smith (3) describes a twig and branch infection of lemon trees caused by *Sclerotinia libertiana* in which "the twigs may become infected through the blossoms or through some injury. The disease is often worse following a cool winter when the tissue is weakened or injured by frost." A similar infection is quite common on frost-injured fig trees in the interior valleys of California. Botrytis also is abundant on such branches and it too is mentioned by Smith as being associated with the lemon disease.

These diseases of the apricot, almond, lemon, and fig are exceptional among the troubles attributed to *Sclerotinia sclerotiorum* in that they attack the aerial parts of the host in a manner which precludes soil-borne

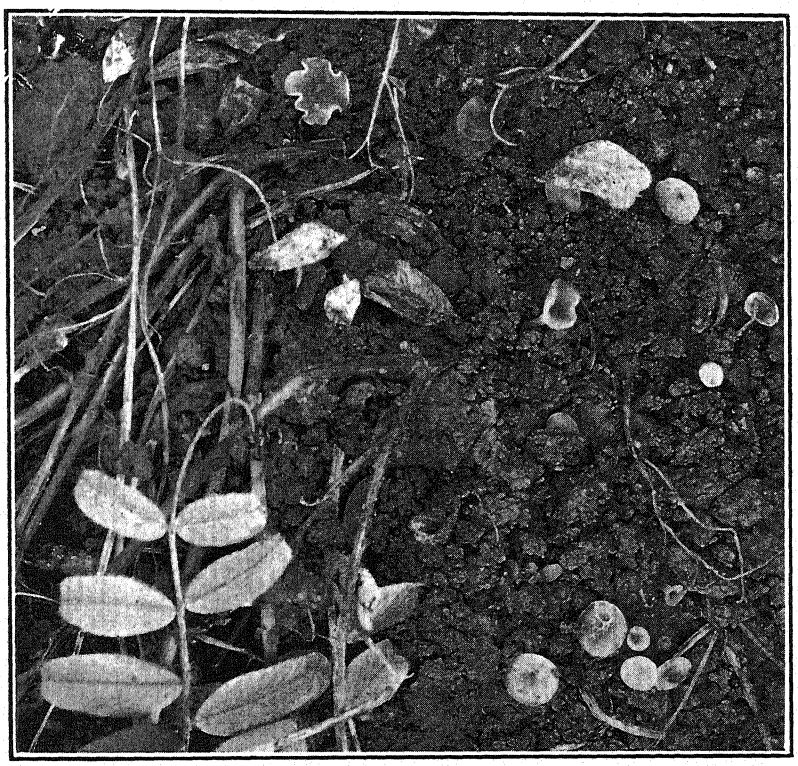


FIG. 5. Apothecia of *Sclerotinia sclerotiorum* in soil beneath vetch cover crop in lemon orchard (after C. O. Smith).

infection by direct mycelial contact. Most diseases caused by this fungus are of the nature of stem or fleshy-part rots originating in the latter manner. In the apricot and almond infection occurs uniformly all over the tree up to a height of many feet above ground. Since in these hosts the fungus attacks only the blossoms and young fruit and has no conceivable method of holding over on the tree in mycelial form, it is the common supposition that infection must originate from spores and that the inception of the disease is due to ascospores originating in apothecia from sclerotia in the soil beneath the tree. Such apothecia, together with the mycelium and sclerotia of the fungus, can actually be found at times in apricot and lemon orchards, particularly where there is a heavy growth of cover crop, weeds, or other vegetation (Fig. 5). In line with this idea advice has commonly been given to apricot growers that orchards be plowed or cultivated before the end of the blooming period in order to break up the surface soil, disturb the growth of the fungus, and prevent the formation of apothecia.

EXPERIMENTAL WORK

As a basis for the successful control of the disease on apricots it seemed desirable to determine the time when the fungus first actually reaches the affected parts. Growers report the complete failure of spraying with any material or at any time to control this disease. The efforts heretofore made consist mainly in the usual spraying for brown rot (Bordeaux mixture in the early blooming stage) together with later applications at the end of the bloom or in the jacket stage (dead calyx still attached to the young fruit). The latter seems the most logical time since the infection of the young fruit very evidently takes place through the dead calyces; it has frequently been demonstrated that the removal of these dried jackets by shaking or hand picking completely prevents infection.

Method.—To determine the time when the fungus first reaches the tree the following method was adopted: blossoms, young fruit, foliage, or other parts to be tested, were gathered at intervals throughout the supposed infection period. Each sample was placed in a separate paper bag, taken at once to the laboratory, and put into a moist chamber on wet filter-paper. The material was left until fungus colonies developed and when any which looked like *Sclerotinia sclerotiorum* appeared transfers were made to culture tubes for positive identification.

The work was started in March, 1929, in the large peach and apricot orchard belonging to the California Packing Corporation, near Merced. The previous year (1928) the Tilton apricots in this orchard had been an almost total loss from green rot.

The first material was collected on March 14, 1929. Up to this time the winter, in general, had been cold and dry and the ground in this orchard

had been very dry until rain fell on March 5 and 12. The soil was covered with an abundant growth of bur clover. The Tilton apricots were just past full bloom. In the majority of the blossoms the very young fruits were enclosed in the calyces with some petals still attached. The trees had been sprayed with Bordeaux mixture about March 2. A careful search revealed no trace of sclerotia or apothecia or mycelium on the soil or vegetation beneath the trees. Comparatively cold, dry weather prevailed during the spring with much frost damage to young fruit.

Table 1 shows that *Sclerotinia sclerotiorum* developed abundantly in moist chamber on apricot and almond material from Merced and Stanislaus counties and on the cover crop from beneath the trees. After observing this the search was widened to include other localities in central California and a variety of host material. Flowers and leaves of various fruit trees and native plants from many different places were collected and placed in moist chambers with the result that *S. sclerotiorum* appeared as one of the commonest fungi occurring upon them. In no case was any development of the fungus visible when the material was gathered and it seemed certain that the organism must have been present in spore form. The original growth was obtained in almost every case only on petals, probably because they formed the most favorable growth medium. Botrytis, apparently of more than one species, was abundant on much of the material but was readily separated from *S. sclerotiorum* in isolation cultures. Figure 3, A, shows typical mycelial growth of *S. sclerotiorum* in the moist chamber coming from young apricot fruits and blossoms with 2 colonies of Botrytis

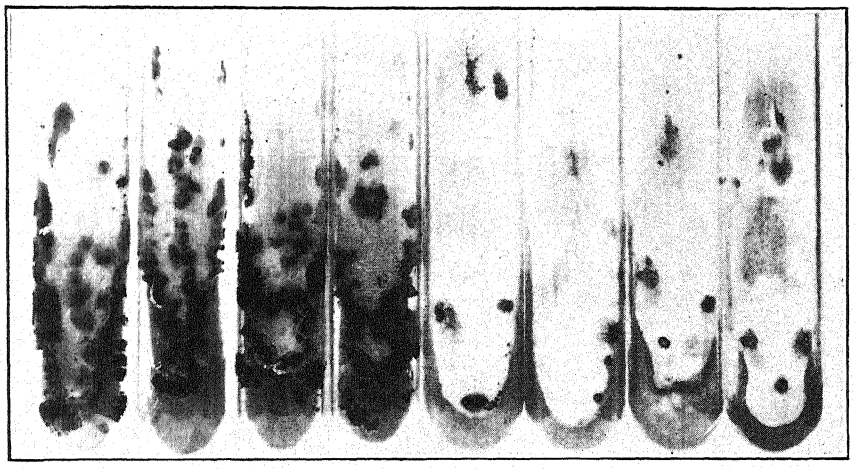


FIG. 6. Cultures of Botrytis (left) and *Sclerotinia sclerotiorum* (right) from young apricots with green rot.

TABLE 1.—*Results of moist chamber tests of apricot and other material for Sclerotinia sclerotiorum*

Lab. no.	Date collected	Locality	Host	Part	Results
50	3/14/29	Merced	Apricot	Flowers	3 isolations Botrytis, 1 *S. sclerotiorum*
51	"	"	Soil and dead leaves from apricot orchard		2 isolations *S. sclerotiorum*, 2 Botrytis
52	"	"	Soil and dead leaves from apricot orchard		No *S. sclerotiorum*
53	"	"	Bur clover[a] in apricot orchard	Green leaves	Botrytis and *S. sclerotiorum* in separate colonies
54	3/15/29	Oakdale	Almond	Flowers	No *S. sclerotiorum*, Botrytis abundant
55	"	"	Soil and dead leaves from apricot orchard		No *S. sclerotiorum*
61	3/18/29	Modesto	Apricot	Flowers	*S. sclerotiorum* abundant
62	3/19/29	Oakdale	Almond	"	No *S. sclerotiorum*, some Monilia
63	3/21/29	Merced	Green undergrowth and dead leaves from apricot orchard		Colonies of Botrytis and *S. sclerotiorum*
64	"	"	Apricot	Flowers	*S. sclerotiorum*
65	"	"	Almond	"	Botrytis and *S. sclerotiorum*
66	3/27/29	Berkeley	Apricot	Young fruits with jackets	Abundant and almost pure growth of Botrytis, no *S. sclerotiorum*
67	"	Yountville	French prune	Flowers	Botrytis and *S. sclerotiorum* abundant
68	"	"	Pear	"	*S. sclerotiorum* doubtful, much Rhizopus
69	"	Napa	Green chickweed[b] from roadside		*S. sclerotiorum*
70A	"	"	Cherry	Flowers	" "
70B	"	"	Pear	"	No *S. sclerotiorum*, much Rhizopus
70C	"	"	Rape	"	*S. sclerotiorum*
75	3/29/29	Merced	Peach	"	" "
76	"	"	Apricot	Young fruits with jackets	" "

[a] *Medicago hispida* Gaertn.

[b] *Stellaria media* (L.) Cyr.

TABLE 1.—*Continued*

Lab. no.	Date collected	Locality	Host	Part	Results
78	3/29/29	Merced	Flowering peach	Flowers	*S. sclerotiorum* very abundant
79	"	"	Peach	"	*S. sclerotiorum*
80	"	"	"	"	Botrytis
81A	"	"	Plum	"	*S. sclerotiorum*
81B	"	"	Apricot	Young fruits with jackets	" "
85	4/3/29	Modesto	"	Young fruits with jackets[c]	No *S. sclerotiorum*
86	"	"	"	" " " " [d]	No *S. sclerotiorum*
87	"	"	"	" " " " [e]	Vigorous growth of *S. sclerotiorum*
88	"	"	Almond	" " " "	Much *S. sclerotiorum*
89	"	Oakdale	"	" " " "	Some Monilia, no *S. sclerotiorum*
90	"	"	"	" " " "	Some *S. sclerotiorum*
94	4/5/29	Berkeley	Crab apple	Flowers	No *S. sclerotiorum*, some Botrytis
98A	4/6/29	"	Cherry	"	" " " " "
98B	"	"	Plum	"	" " " " "
100	4/8/29	"	Barberry	"	" " " " "
101	4/10/29	Sebastopol	Apple and pear	"	No *S. sclerotiorum*, much Rhizopus
102	"	Petaluma	Annual wild flowers		*S. sclerotiorum* very abundant
106A	4/11/29	Merced	Apricot	Young fruits without jackets	*S. sclerotiorum*
106B	"	"	"	Jackets from last	No *S. sclerotiorum*, Botrytis abundant
108	"	"	Green bur clover from apricot orchard		*S. sclerotiorum*
109	"	Morganhill	French prune	Flowers	" "
110	"	Merced	Peach	Young fruit	No *S. sclerotiorum*
115	4/18/29	Brentwood	Apricot	Young fruit killed by frost	*S. sclerotiorum*
116	4/19/29	Berkeley	"	Young fruit	No *S. sclerotiorum*, much Botrytis
117	4/20/29	"	Cherry	Young fruit in jackets	No *S. sclerotiorum*
130	5/18/29	Merced	Apricot	Young fruit and leaves	" " "
131	"	"	"	Young fruit from ground	" " "

[c] Sprayed with Bordeaux mixture and oil in jacket stage.

[d] Sprayed with lime sulphur in jacket stage.

[e] No spraying.

TABLE 1—*Continued*

Lab. no.	Date collected	Locality	Host	Part	Results
132	5/18/29	Merced	Green bur clover from apricot orchard		*S. sclerotiorum* abundant
133	"	"	Dead and moldy bur clover from apricot orchard		No *S. sclerotiorum*
134	"	Delhi	Wild primrose[f]	Flowers	" " "
135	"	"	Wild lupine[g]	"	" " "
136	5/24/29	Rocklin	California poppy[h]	"	" " "
141	6/19/29	Sebastopol	Native wild plants	"	" " "
142	"	Napa (same as 69)	California poppy	"	" " "
144	6/24/29	Modesto	Peach	Fruit deformed by frost	No *S. sclerotiorum*, white mycelium of Fusarium
165A	2/12/30	Merced	Apricot	Twigs with unopened buds	No *S. sclerotiorum*
165B	"	"	Green grass from apricot orchard		" " "
165C	"	"	Dead leaves and grass from apricot orchard		" " "
165D	"	"	Almond	Flowers and buds	" " "
166A	2/22/30	Atascadero	"	Flowers	" " "
166B	"	Morganhill	"	"	Much *S. sclerotiorum*
166C	"	"	Weeds in prune orchard		No *S. sclerotiorum*
167A	2/28/30	Turlock	Apricot	Flowers	*S. sclerotiorum*
167B	"	Byron	Yellow flowered annual composite	Whole plant with flowers	No *S. sclerotiorum*
167C	"	Merced	Apricot	Flowers	*S. sclerotiorum*
167D	"	"	"	"	" "
167E	"	"	"	"	" "
167F	"	"	"	"	" "

[f] Oenothera.

[g] Lupinus.

[h] *Eschscholtzia californica* Cham.

TABLE 1—*Continued*

Lab. no.	Date collected	Locality	Host	Part	Results
167G	2/28/30	Escalon	Apricot	Flowers	*S. sclerotiorum*
167H	"	Oakdale	Almond	"	" "
168A	3/7/30	Morganhill	Melilotus	"	" "
168B	"	"	Wild radish[i]	"	" "
168C	"	"	Almond	"	" "
168D	"	"	Mustard[j]	"	" "
169A	3/22/30	Collegeville	"	"	" "
169B	"	Turlock	California poppy	"	" "
169C	"	"	Apricot	"	*S. sclerotiorum* abundant
169D	"	Atwater	Peach	"[k]	" " "
170A	4/1/30	Napa	California poppy	Flowers	" " "
170B	"	"	Prune	"	" " "
171A	4/4/30	Merced	Apricot	Dried jackets	*S. sclerotiorum* abundant on every calyx
171B	"	Escalon	"	Leaves	No *S. sclerotiorum*
172	4/14/30	Berkeley	Cherry	Calyces	No *S. sclerotiorum*, much Botrytis
173	4/29/30	Merced	Mustard	Flowers	No *S. sclerotiorum*

[i] *Raphanus sativus* L.

[j] *Brassica campestris* L.

[k] Sprayed with Bordeaux in bud.

beginning to appear. This photograph was taken 48 hours after the material was put into the moist chamber.

Typical cultures of the 2 fungi Botrytis and *Sclerotinia sclerotiorum* are shown in figure 6. Botrytis is grayish brown with typical conidia production (varying in abundance) and thin membranous sclerotia inseparably attached to the medium. Sclerotinia shows pure white mycelium, total absence of conidia, and round meaty sclerotia separating cleanly from the substratum. *S. sclerotiorum* was never obtained on material from Berkeley (Nos. 66, 94, 98A, 98B, 100, 172). The number and kind of samples tested appeared sufficient to give some significance to this result. Flowers of pear and apple (Nos. 68, 70B, 101) never yielded *S. sclerotiorum* but always a great abundance of Rhizopus. It does not appear possible, however, that any host specificity as to the occurrence of the fungus could have existed in light of its universal distribution on other material in the same vicinity. It is more probable that certain materials were more favorable than others to the growth of the fungus, even though the spores were present on all. Mustard blossoms and flowers of the California poppy (Eschscholzia) seemed to furnish a particularly favorable medium since such material from different localities usually developed an abundant growth of *S. sclerotiorum*. The various specimens of these plants were gathered in all sorts of places, orchards, alfalfa fields, and bare land, and along roadsides.

The fungus was not obtained from fruit trees after the latter part of April, when it began to become less abundant, but was recovered from green bur clover on May 18. This as usual was on sound, green leaves which showed no visible sign of infection before being placed in the moist chamber.

No rain fell during May but there was considerable precipitation during the first half of June. The last test was on June 24. Not a single case of green rot or any visible natural development of *Sclerotina sclerotiorum* was observed in 1929.

In 1930 the work was reopened on February 12. Almonds were in bloom, but on the Tilton apricots in the Merced orchard the flowers had not yet opened. No cover crop had been planted in this orchard and the ground had recently been cultivated so that it was completely bare of vegetation, except a little grass and dead leaves around the base of each tree. No rain had fallen for two weeks and the soil was quite dry. No sign of the mycelium, sclerotia, or apothecia of *Sclerotinia sclerotiorum* could be found beneath the trees and the conditions were entirely unfavorable for any such development. The first sample taken for culturing consisted only of twigs and buds, no blossoms having opened. It could hardly be expected that the fungus would develop upon such material even if spores were

present. The almond blossoms, however, gathered on the same day in the same locality, constituted a very favorable medium, but these did not develop the typical organism. Almond blossoms gathered at Atascadero (San Luis Obispo County) on February 22 did not develop the fungus, but similar material collected the same day at Morganhill (Santa Clara County) gave a very abundant growth. This came from an orchard with perfectly bare, dry soil and with conditions in every way most unfavorable to the growth of *S. sclerotiorum* in the ground under the trees. Commencing February 28 and continuing until April 4, a series of 18 samples representing a wide variety of material growing under various conditions in Merced, Stanislaus, San Joaquin, Contra Costa, Napa, and Santa Clara counties did not fail to develop an abundance of the fungus in any instance. In the case of sample No. 168A (from a heavy growth of Melilotus under prune trees at Morganhill) *S. sclerotiorum* was growing quite abundantly in places on the dense mass of vegetation in the orchard. This was the only case during this entire investigation where visible growth of the fungus was found under natural conditions. Even here, however, perfectly sound, individual, separate leaves taken at a height of 2 or 3 feet from the ground developed *S. sclerotiorum* almost as quickly and abundantly as did foliage from the thick mat of growth near the soil where the fungus growth was visible in the orchard. This indicated the presence of spores distributed abundantly all over the vegetation as well as the contact mycelium near the ground. No apothecia were found in this orchard.

No case of green rot was found during 1929 and 1930. No apothecia were found in the soil, and, with the exception just noted (No. 168A), no natural infection with *Sclerotinia sclerotiorum* on any host was observed.

Note: In 1931 moist-chamber testing of blossoms was commenced on January 20 and continued to March 10. It was a season of humid, mild weather and the fungus *S. sclerotiorum* was found to be universally present in extreme abundance on all sorts of vegetation in central California from Napa to Merced County. Flowers of fruit trees and annual plants were apparently covered with spores and the fungus developed profusely in almost pure culture whenever such material was put into moist chambers. No such development occurred, however, on the flowers in nature and the presence of the fungus would never have been suspected without the moist-chamber test.

During the current season apothecia of *S. sclerotiorum* were found in abundance, developing from old sclerotia in the soil among thick vegetation, particularly in fields of yellow mustard, *Brassica campestris* L. This is one of the commonest winter-blooming plants in central California and covers

thousands of acres of fields, vineyards, and orchards with solid sheets of yellow. From this season's experience it would appear that such fields of this or other favorable vegetation form the breeding ground of the fungus. Mycelium and sclerotia develop each year during the latter part of the winter on the plant remains in the soil. The sclerotia remain over summer and start to develop apothecia with the next winter's rains, thus producing successive crops of ascospores which fill the air of the whole vicinity. Whether the vegetation upon which the fungus develops and upon which the production of apothecia and ascospores depends is directly beneath the trees or at some distance is of little consequence.

DISCUSSION

The foregoing results indicate that:

1. In the green rot of the apricot and almond, infection with *Sclerotinia sclerotiorum* must take place by means of spores.

2. The production of such spores has no relation to the soil directly beneath the trees, the presence or absence of a cover crop, or similar conditions.

3. The fungus has no specificity for or limited occurrence on the apricot, almond, or other hosts on which infection may occur, but in the early part of 1929 and 1930 was present in great abundance on all sorts of vegetation.

4. The abundant presence of spores of *S. sclerotiorum* on vegetation has no correlation with the development of the fungus on vegetation or soil directly beneath.

5. Spore production and distribution must commence as early as February, previous to the time when growth of the fungus on soil and surface vegetation occurs. Spores must continue to be produced and distributed throughout the spring, since much of the material tested in the latter part of the season had not yet developed in the earlier part.

6. The distribution of the fungus is not effected by ascospores produced in easily found apothecia in the soil in close proximity to the final resting place of the spores.

The whole situation indicates the occurrence of some spore form of *Sclerotinia sclerotiorum* which is very abundantly produced and generally distributed throughout the spring season, regardless of whether conditions are favorable for the germination of spores and the development of a disease like green rot. In two seasons, at least (1929 and 1930), when no green rot appeared and almost no development of *S. sclerotiorum* on annual vegetation was observed (sample No. 168A being the only exception), the fungus was universally present when given an opportunity to develop in a moist chamber.

Relation to Botrytis: The fact that Botrytis is so commonly associated with *Sclerotinia sclerotiorum* in almost all cases of its occurrence and has even in some cases (Nixon and Curry (2), Smith and Smith (5)) been thought to be the cause of green rot raises the old question of the possibility of this being a conidial stage of *S. sclerotiorum.* If this were true the whole question under discussion would be solved. Gäumann (1, p. 325) says: "The economically important representatives of the subgenus *Eusclerotinia*, as *S. Fuckeliana, S. Libertiana, S. Trifoliorum* and the various Sclerotinias on monocotyledonous bulbs, in their choice of hosts are much less specialized than those of the subgenus *Stromatinia* . . . . During the summer they kill the infected organs, developing in them the sclerotia in which they over-winter. Their conidia arise singly on characteristically formed conidiopores and hence, in contrast to those of *Stromatinia,* are placed in *Botrytis* rather than in *Monilia.* As the cultural determination of the perfect form is rarely possible, one generally places them, especially those of the *S. Fuckeliana* and *S. Libertiana* groups, in the collective species *Botrytis cinerea.*" In the same connection these authors reproduce an illustration of Botrytis by the writer with the legend "*Sclerotinia sclerotiorum.* Conidial stage *Botrytis cinerea* (after R. E. Smith, 1900)." Inasmuch as this drawing was published in an article attempting to prove that Botrytis is *not* the conidial stage of *S. Libertiana* (*sclerotiorum*), the inference is a somewhat ambiguous one.

In the investigation here reported no difficulty was ever experienced in definitely separating Botrytis and *Sclerotinia sclerotiorum* in culture, as stated on page 413 and illustrated in figure 6, even though their simultaneous occurrence here was typically common. The writer has, furthermore, been unable to find any literature or experimental evidence to substantiate the statement of Gäumann or to disprove the writer's own conclusion, published in 1900, that "*Sclerotinia libertiana* and *Botrytis cinerea* have no connection whatsoever with each other, and that the former species has no conidial stage of this type." There appear, therefore, to be no grounds for accepting the attractive hypothesis that the distribution of *S. sclerotiorum* takes place by means of a conidial stage of the Botrytis type.

Microconidia (spermatia, pycnospores): There is occasionally found, on the mycelium or sclerotia of *S. sclerotiorum*, an abundant production of minute, acrogenous microconidia. These at times have been seen to germinate and make a feeble growth. That they have any important function, however, in the reproduction of the fungus is extremely doubtful.

Ascospores: The usual conception of the life history of *S. sclerotiorum* is that already indicated, that the sclerotia, formed one year, lie over winter and reproduce the fungus the following year by the development of

ascospores or mycelium. In the case of the lemon the matter is well expressed by C. O. Smith (3, p. 245) as follows: "The vegetative stage is the white, cotton-like growth. . . . This growth has no spores, but can spread rapidly and has the power to infect . . . by . . . contact. . . . In this white growth, numerous black bodies known as sclerotia are developed. . . . After these bodies have gone through a period of rest and the favorable rainy, cool weather of the autumn and winter has come, they revive and become active. The sclerotia may then send out a fresh growth of mycelium, the vegetative stage, which could easily infect dead organic matter or the weaker plant growth of weeds or cover crops. Here after a time new sclerotia are again formed. From the sclerotia in the soil the spore stage (ascospore) is formed under the cover crops in the lemon orchards. . . . Some of these spores are carried by the wind to the fruit and twigs . . . where under favorable conditions they germinate and produce the vegetative stage. The apothecial stage in California begins to appear about the first of October and continues to be formed during the winter and spring. After the cover crops of the orchards are plowed under and cultivation begins, few apothecia (spore stage) are thought to be produced, for this stage the fungus requires moist, shaded conditions, such as are best found under cover crops during the rainy season."

On the basis of present knowledge the dissemination of wind-borne ascospores from sclerotia in the soil furnishes the only explanation of the distribution of this fungus on the aerial parts of plants. To account for the origin of such ascospores, in such abundance and general distribution as have been shown to be the case, it seems necessary to seek some more obscure and distant source than the soil beneath the trees or plants.

SUMMARY

The "green rot" disease of apricots and almonds, an infection of young fruits through adherent, dried calyces, occurs in a very sporadic manner in central California.

The usual cause of the disease is the fungus *Sclerotinia sclerotiorum*. *Botrytis cinerea* Pers. is commonly associated with affected material and, in some instances, has been considered to be the cause of the disease.

The common supposition has been that infection is caused by wind-borne ascospores originating in the soil beneath the trees from sclerotia produced by the fungus growing on cover crop or other surface plants.

Moist-chamber tests of blossoms of apricot and other fruit trees, as well as those of many annual plants, from many places in central California, showed that from about February 15 to June 15 this fungus was universally present on vegetation, presumably in some spore form. This was true

during two seasons (1929 and 1930) when no green rot appeared, when no development of *Sclerotinia sclerotiorum* (save in one minor instance) on surface-growing plants was observed, and in situations where vegetative growth of this fungus was extremely unlikely.

No evidence was found to suggest that *S. sclerotiorum* possesses a conidial stage of Botrytis or any other type.

It would appear that the aerial distribution of this fungus is much more general than has hitherto been suspected.

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PLANTS AFFECTED BY FIRE BLIGHT[1]

H. E. THOMAS AND H. E. THOMAS

The plants known to be affected by fire blight, caused by *Bacillus amylovorus* (Burr.) Trev., already comprise an imposing group and have been reviewed by Snow (7), Rosen and Groves (6), and Pierstorff (4). At first glance it might appear that the list for North America is fairly complete. The cultivated plants of California, however, include a number of rosaceous trees and shrubs, some of them recently introduced, which do not seem to have been studied in relation to their susceptibility to fire blight.

It has been observed by various workers in California during the past several years that species of Cotoneaster and Pyracantha are affected by a disease assumed to be fire blight, although the writers have found no published record of experimental proof for any species of these genera save the early report by Arthur (2) from New York for *P. coccinea*.[2,3]

The work reported in this paper was initiated primarily to study the susceptibility of species of Pyracantha and Cotoneaster and was later expanded to include a number of other readily available plants. A few previously tested plants were included and certain others for which the evidence seemed inadequate. A number of inoculations were made on potted plants in the greenhouse at Berkeley, California, during the winter and spring of 1929–30. The remainder were field-inoculation tests at the University of California Deciduous Fruit Station, San José.

Of the plants used in the field, the species of Cotoneaster, Heteromeles, Kerria, Photinia, Pyracantha, Raphiolepis, and Spiraea were planted on February 5, 1930. The remaining species and varieties were planted one year earlier. All the plants were making satisfactory growth at the time of inoculation with a few exceptions, which will be noted. Most of the inoculations on these were made in May, 1930.

In consideration of the possible existence of pathogenic strains of the fire-blight organism, eight cultures were used for the majority of the inoculations. These represented pear, apple, Cotoneaster, and Pyracantha and, geographically, California, Michigan, New York, and South Carolina. They were composited immediately before they were used and no experi-

[1] Submitted for publication August 22, 1930.

[2] In the names of plants Rehder (5) is accepted as the guide for the most part.

[3] A typewritten thesis by Adrian C. Wilcox (Relation of miscellaneous plants to the pear industry. Thesis for the degree of Bachelor of Science in the University of California. May, 1920. Unpublished) on file in the Division of Plant Pathology of the University of California records successful inoculations on a single shoot each of *Pyracantha coccinea, Cotoneaster pannosa,* and *C. microphylla.*

ments designed to distinguish differences in pathogenicity between the cultures were undertaken. Inoculations were usually made from bouillon cultures with dissecting needles ground to a fine point.

The tips of growing shoots were inoculated in most instances. In a few cases in which these failed, later inoculations were made on older stems. Since none of the latter were successful, they will not be treated separately. In a single instance (*Cotoneaster Harroviana*) flower clusters were inoculated.

RESULTS OF INOCULATION EXPERIMENTS

It is highly desirable to place on record some evidence of the degree to which susceptible plants may be affected. This has been attempted in the results of some of the inoculations summarized in table 1. The data obtained from inoculations at any given date, however, are only approximately comparable to those of another date, though they be taken from the same planting and at short time intervals. Infection and the subsequent course of disease seem to be very intimately related to conditions of growth rate of the plant and to temperature and humidity during and after the incubation period. There is, moreover, the probability that certain plants may escape inoculation in nature more often than do others.

The problem of definitely placing a given species as to the degree of susceptibility is further complicated in the genera Cotoneaster and Pyracantha by the fact that considerable variation exists within certain species. This is not surprising in view of the common practice among nurserymen of growing these plants from seed. Specific instances of distinct differences in response to inoculation by plants of the same species from different sources will be noted later.

Infection experiments in the field probably afford a more nearly exact basis for predicting the response of plants to infection in nature than experiments in the greenhouse. The latter will be reported therefore only when some special interest attaches to them. Since more particular attention was given to the genera Cotoneaster and Pyracantha, these will be treated in somewhat greater detail.

Judging from parallel inoculations on a susceptible variety of apple (White Astrachan), it is concluded that the conditions under which the inoculations on plants of these genera were made were only moderately favorable for infection.

COTONEASTER MED. Several species of Cotoneaster have gained wide popularity as ornamental shrubs in California. There may therefore be considerable direct loss from blight, due to the death in whole or in part of the shrubs themselves, as well as a considerable menace to neighboring fruit trees. The results of inoculation experiments on the following fourteen species are presented in table 1.

Cotoneaster acuminata Lindl.
" *adpressa* Bois
" *Dammeri* Schneid. var. *radicans* Schneid. (F. P. I. 52677)[4]
" *Dielsiana* Pritz. var. *elegans* Rehd. and Wils.
" *Francheti* Bois
" *frigida* Wall.
" *Harroviana* Wils. (In part F. P. I. 72794)
" *horizontalis* Decne.
" *lactea* (F. P. I. 62569)
" *microphylla* Wall.
" *pannosa* Franch.
" *prostrata* Baker (F. P. I. 56304)
" *salicifolia* Franch. var. *floccosa* Rehd. and Wils. (In part F. P. I. 62256)
" *Simonsii* Bak.

For those species which were inoculated only in the greenhouse, the extent of invasion by the organism is not tabulated, since these tests are not considered comparable with those made in the field.

Three preliminary tests on a small scale were made with *C. adpressa.* In two of these a small amount of infection resulted. In a fourth test 20 shoots were inoculated on 2 plants. After 12 days, 14 of these were blighted. The organism penetrated to a maximum distance of about $1\frac{1}{2}$ inches from the point of inoculation but in most cases was checked at about $\frac{1}{2}$ to $\frac{3}{4}$ inch. All these tests were made in the greenhouse.

Two small plants of *C. Dammeri* var. *radicans* were available for greenhouse study. Five inoculations were made on each of two dates. Vigorous infection resulted in all of these and invasion was rapid until all the new growth was involved.

In addition to the field inoculations of *C. Francheti,* recorded in table 1, seedlings were inoculated in the greenhouse on four different dates. No symptoms developed on any of these.

Late in the season of 1929 at Chico, California, *C. frigida* was observed affected by blight in the larger branches as well as in the smaller shoots. An attempt to isolate the organism was unsuccessful, but the failure may be accounted for by the exceedingly dry weather in that region throughout the summer and autumn. From cuttings taken on the University campus at Berkeley a single plant was obtained in the greenhouse. Five days after inoculation distinct symptoms were evident on the plant and in 11 days all the new growth was involved. In striking contrast is the resistance

[4] The writers are indebted to the Office of Foreign Plant Introduction of the Bureau of Plant Industry, United States Department of Agriculture, for some of the plants used in these tests. The corresponding F. P. I. number is shown in such cases.

TABLE 1.—*Results of inoculation of Cotoneaster and Pyracantha with the fire-blight organism, Bacillus amylovorus*

Name of plant	No. of plants	No. of inoculations	No. of infections	Average distance traversed by organism (inches)[a]		Shoots invaded beyond current season's growth
				In 30 days	In 55 days	
C. acuminata	5	30	0			
C. adpressa	2	20	14	[b]		
C. Dammeri var. *radicans*	2	10	10	[b]		
C. Dielsiana var. *elegans*	5	30	0			
C. Francheti	5	30	0			
C. frigida	5	30	0			
C. Harroviana	5	30	2	[c]		
C. horizontalis	5	20	4	3.2	4.7	3
C. lactea	2	25	0	[b]		
C. microphylla	5	20	2	0.3	0.3	
C. pannosa	5	20	14	7.3	12.2	8
C. prostrata	2	20	1	0.5	0.5	
C. salicifolia var. *floccosa*	7	28	8	5.5	7.2	2
C. Simonsii	5	30	0			
P. angustifolia	5	20	13	5.6	7.5	9
P. coccinea	5	30	2	0.3	0.3	
P. coccinea var. *Lalandii*	5	20	5	3.4	3.4	
P. crenulata	5	20	12	5.5	7.4	6
P. crenulata var. *kansuensis*	5	20	12	5.8	7.8	7
P. crenulata var. *Rogersiana*	5	20	8	2.8	3.2	1
P. Gibbsii var. *yunnanensis*	5	20	4	8.8	11.0	4
P. formosiana	5	20	13	2.9	3.9	1
Apple (White Astrachan)	2	20	8	4.7	5.8	1

[a] Average from the shoots which actually became infected.

[b] Inoculated in the greenhouse, only.

[c] Both infections in flower clusters.

shown by the plants from another source inoculated at San José. The plants of this species from different sources vary considerably in appearance as well as in susceptibility to blight.

Plants of *C. Harroviana* were rather severely blighted in a garden at Berkeley in 1929 and the fire-blight organism was obtained from these in pure culture. Nevertheless, 13 inoculations on plants (F. P. I. 72794) in

the greenhouse on three different dates failed to produce any symptom of disease. It will be noted further that inoculations in the field on plants from another source produced at most a relatively mild infection. Here, apparently, is another case of variation as to susceptibility within a species.

PYRACANTHA ROEM. (FIRETHORN). The position of this genus in popularity among the ornamental shrubs in California is similar to that of Cotoneaster. The species and varieties used in these tests are listed below and the results of inoculations are presented in table 1.

Pyracantha angustifolia Schneid.
" *coccinea* Roem.
" " " var. *Lalandii* Dipp.
" *crenulata* Roem. (F. P. I. 55997)
" " " var. *kansuensis* Rehd. (F. P. I. 40736)
" " " " *Rogersiana* A. B. Jacks (F. P. I. 72814)
" *Gibbsii* A. B. Jacks var. *yunnanensis* Osborn
" *formosiana* (?)

Pyracantha angustifolia is one of the most attractive of the shrubs grown in California particularly during the winter months. Unfortunately, it also is one of the most susceptible species of this genus. Natural infections were abundant in Berkeley during the summer of 1930 and in one planting the disease was severe.

Pyracantha Gibbsii var. *yunnanensis* has exhibited rather marked variation in response to inoculations. It will be noted (Table 1) that the extent of invasion following inoculations in the field is greater than in any other species of the genus, although the number of infections was small. In contrast are the results of a small number of inoculations on seedlings from three different sources in the greenhouse. Although these inoculations were made on six different dates from January to July, the plants from two sources developed no symptoms, whatever. A single plant from the third source became blighted apparently as readily as did the plants in the field.

CHAENOMELES LINDL. Blighted fruits of *Chaenomeles sinensis* Koehne were received from a garden in Berkeley where scions of the species had been grafted on the common quince, *Cydonia oblonga* Mill. (These fruits were placed in a moist chamber and soon became entirely involved by the disease). The symptoms were typical of fire blight, which was also present on the shoots and blossom clusters of the common quince. The bacterial exudate from the fruits of *C. sinensis* was used to inoculate 5 seedling apple shoots. At the end of 4 days all of these bore typical symptoms of fire blight.

DIOSPYROS L. (PERSIMMON). In the first experiment with this genus, ten inoculations were made in the field on a single plant of *Diospyros Lotus* L. After 8 days all the inoculated shoots showed some blackening around

the wounds made by the inoculating needle. At the end of 14 days one shoot was blighted to a distance of 4 inches from the point of inoculation and bore a small amount of bacterial exudate. At the same time the discoloration had spread on 2 other shoots for a short distance ($\frac{1}{4}$ to $\frac{1}{2}$ inch). No further development of symptoms was noted, however, after this time. Thirty additional inoculations were made on shoots of this species at later dates. Only slight symptoms developed in 3 of these.

Diospyros Kaki L. was inoculated on two occasions in a total of 15 shoots. Some evidence of infection was seen on 2 shoots but this was slight and the species is consequently left in the doubtful class.

Thirty inoculations were made on *D. virginiana* L. without the production of any symptoms.

HETEROMELES ROEM. Waite (8) in 1906 observed the typical symptoms of fire blight on *Heteromeles arbutifolia* Roem., the common Toyon or Christmas berry, at Vacaville, California. This author states that "several of the twigs contained the living bacilli" but, apparently, he made no cultures or infection experiments. Although Waite's conclusion has been supported by the observations of a number of workers since that time, it has seemed desirable to make inoculations on this species. Seedlings were inoculated in the greenhouse on three dates. Infection resulted on each occasion, although the plants were small and not in so vigorous condition as is desirable. Eleven inoculations were made on 5 poorly growing plants in the field. Seven infections resulted, involving most or all of the new growth in each case. In view of all the evidence it cannot be questioned that this species may be and frequently is affected by fire blight in nature. The growth condition of the plants used in these experiments does not permit of any attempt to estimate exactly the degree of susceptibility of the Toyon as compared with the other plants studied.

KERRIA DC. *K. japonica* DC. was inoculated on three occasions in the greenhouse and once (on 20 shoots) in the field. No trace of a symptom resulted in any instance.

PHOTINIA LINDL. Pierstorff (4) in a paper now in manuscript records successful infection experiments with *P. villosa* DC. The writers have tested (in the field) *P. serrulata* Lindl. and a similar form sold under the name *P. dentata* for which they have found no authority. At the time of the first inoculations the plants were making rather poor growth. No infection resulted from 16 inoculations on each sort. Later when the growth condition of *P. serrulata* was somewhat better, 20 inoculations were made on this species and 5 on *P. dentata*. No certain symptoms were produced.

PRUNUS L. While fire blight has been recorded on several species of this genus, there is distinctly less susceptibility here than in the subfamily Pomoideae of the rose family. It is desirable, however, to make further

inoculations in this and related genera in order that a more nearly balanced view may eventually be obtained of the relative susceptibility of the plants which may be affected by the fire-blight disease. The complete list of Prunus species used in these tests is given below.

Prunus allegheniensis Porter	Alleghany plum
" *americana* Marsh.	Plum
" *Armeniaca* L.	Apricot
" *avium* L.	Cherry (Bing variety)
" *Besseyi* Bailey	Western sand cherry
" *bokhariensis* Royle	Plum (India)
" *cerasifera* Ehrh.	Myrobalan (cherry plum)
" *Cerasus* L.	Cherry (morello)
" *communis* Arcang.	Almond
" *dasycarpa* Ehrh.	Purple apricot
" *domestica* L.	Agen or French prune
" *hortulana* Bailey	Hortulana plum
" *ilicifolia* Walp.	Holly leaf cherry
" *insititia* L.	St. Julien G. and Black Damas C.
" *mahaleb* L.	Mahaleb cherry
" *mira* Koehne	
" *mume* Sieb. and Zucc.	Japanese apricot
" *Persica* Batsch	Peach (a flowering variety)
" *salicina* Lindl. X *P. cerasifera* Ehrh. (Myrobalan)	Methley plum
" *serrulata Lannesiana* (Carriere) Koehne	A flowering cherry
" *Simonii* Carr.	Apricot plum
" *tomentosa* Thunb.	Manchu cherry

Since relatively few infections were produced on the plants of this genus, the results are not presented in tabular form.

From 5 to 50 (usually ten) inoculations were made on each species. Parallel inoculations on apple demonstrated that the conditions at the time these were made were quite favorable for infection and more so than was the case with the inoculations on Cotoneaster and Pyracantha. In some instances effects were produced, such as gumming or discoloration at the point of inoculation, which may have been due to the presence of the organism, but only those species that exhibited more definite symptoms will be treated in the following paragraphs.

All of the inoculations with the exception of those on *P. ilicifolia* were made in the field on May 23, 1930, and the final notes were made on July 1.

Ten shoots of *P. allegheniensis* were inoculated. At the end of the experiment 5 of these bore some symptoms of blight. Invasion was very limited, however, varying from $\frac{1}{4}$ to $\frac{3}{4}$ inch.

Two varieties of apricot, Tilton and Royal, were tested. On the Tilton there was a small amount of discoloration and bacterial exudate but none of the tips of shoots was entirely killed. On the Royal variety 7 of the 10 inoculated shoots were killed at the tip or on one side of the shoot to distances of $\frac{1}{4}$ to $1\frac{1}{4}$ inches.

Ten shoots were inoculated on 2 plants of *P. Besseyi*. Nine of these suffered some injury, mostly less than 1 inch in extent. One of them, however, blighted to a distance of 3 inches in 11 days, accompanied by the typical bacterial exudate.

Fifty inoculations were made on 5 selections of myrobalan plums, 10 on each lot. On 2 of these lots, both of the red-leaf type, distinct symptoms were produced. One collection listed in the station records as Davis myrobalan 2567 yielded 8 infections ranging in extent from $\frac{1}{2}$ to $2\frac{1}{2}$ inches. The other, propagated from a chance myrobalan seedling, became slightly affected in 4 of the 10 inoculated shoots.

Prunus dasycarpa became infected in 3 of 10 inoculated shoots, with a maximum penetration of $1\frac{1}{2}$ inches.

About 30 inoculations were made on seedlings of *P. ilicifolia* in the greenhouse on 5 different dates. Following one of these there was some killing of the tips of shoots, but, since the results of all the other tests were entirely negative, it is concluded that these plants probably are highly resistant to, if not immune from, fire blight.

Two lots of *P. mume* were inoculated. On one of these, 2 of 10 inoculated shoots showed a small amount of killing but scarcely enough to more than place this species in the doubtful class.

The hybrid Methley plum developed 8 infections from 10 inoculations, with a maximum invasion of 1 inch.

Prunus Simonii became infected in 8 of 10 inoculated shoots, but the lesions ceased development at $\frac{1}{4}$ to $\frac{3}{4}$ inch in length. Paddock (3) observed what he believed to be natural infection on this species in Colorado, in 1903, but apparently made no cultures or inoculation experiments.

PYRUS L. (PEAR AND APPLE). *Pyrus Malus* L. var. *Niedzwetzkyana* Asch. and Graebn., a red-leaf, ornamental crab apple, was inoculated in 6 shoots. Invasion was slow but definite and finally resulted in killing on 4 shoots to an extent of $1\frac{1}{4}$ to $6\frac{1}{2}$ inches.

Five inoculations were made on a single young tree of *P. prunifolia* Willd., plum-leaf crab, in the field. Infection resulted in 3 shoots with a maximum invasion of 3 inches. In a later test, all of 10 inoculated shoots were blighted to a distance of $\frac{1}{2}$ to $3\frac{1}{4}$ inches from the point of inoculation.

The hybrid kaido crab, *P. micromalus* Bailey, was inoculated in 9 shoots. Only 2 of these became infected but these were eventually killed to 4 and 12 inches from the point of inoculation.

RAPHIOLEPIS LINDL. Two species of these evergreen shrubs were tested at San José.

Five plants of *R. indica* Lindl. in only moderate vigor were inoculated in 21 shoots. Twelve of these developed some symptoms and several were invaded for 2 to 5 inches. In view of the growth condition of the plants, it is concluded that a rather marked susceptibility inheres in this species.

The 5 plants of *R. umbellata* Mak. were small and made poor growth throughout the period covered by these experiments. Twenty-one inoculations were made on these. There was some evidence of invasion in a few shoots, but further inoculations are desirable before any definite conclusions are drawn for the species.

SPIRAEA L. Twenty inoculations were made on each of *S. cantoniensis* Lour., *S. prunifolia* Sieb. and Zucc., and *S. Vanhouttei* Zabel. No infection resulted. These inoculations were repeated later on the same plants. Some injury resulted on *S. Vanhouttei* and *S. cantoniensis* but it was scarcely sufficient to be conclusive. No symptoms were produced on *S. prunifolia*.

A blight of *Spiraea* sp. has been reported as fire blight from Maryland (1) (without experimental proof), and Rosen and Groves (6) reported symptoms of blight resulting from inoculations on detached twigs of this species.[5]

DISCUSSION

An exact account of the degree to which susceptible plants under natural conditions may be affected by fire blight must await more extensive observations, even for plants which have long been recognized as affected by this disease. For example, apricot, cherry, plum, and prune have been on record for many years as susceptible, yet few observations seem to have been made on fire blight among these plants in the orchard. Relatively little time has been given by the writers to a search for natural infection of the susceptible plants here reported for the first time. Such infections have been seen, however, presenting the typical symptoms of fire blight on *Cotoneaster frigida, C. Harroviana, C. pannosa, Pyracantha angustifolia, P. coccinea Lalandii, P. crenulata, P. crenulata kansuensis,* and *P. crenulata Rogersiana*. The fire-blight organism was isolated from blighted specimens of 3 of these species.

It is inferred by comparison with parallel inoculations on apple that *Cotoneaster pannosa, Pyracantha angustifolia, P. crenulata, P. crenulata kansuensis,* and *P. Gibbsii yunnanensis* exhibit a degree of susceptibility approaching that of apple and pear. The limited tests with *C. Dammeri radicans* indicate a rather marked susceptibility in this species.

[5] Since this paper was written, Groves has presented conclusive evidence of the susceptibility of *S. Vanhouttei.* (U. S. Dept. Agr. Plant Dis. Rep. 14: 133. 1930).

It is probable that the blight organism will be found to survive the winter in plants of several of these species, since cankers not uncommonly extend into branches several years old. The menace to fruit trees, however, offered by these shrubs, is not so great as it may appear, since most of the susceptible species of Cotoneaster and Pyracantha come into blossom distinctly later than the pear and apple. This is also true of the Toyon.

The existence of even a slight degree of susceptibility in a genus so remotely related to the rose family as Diospyros (ebony family) suggests that the list of susceptible plants may yet be far from complete. It is of interest, however, to note that within a single susceptible species (*Cotoneaster frigida*) or variety (*Pyracantha Gibbsii yunnanensis*) plants may be found that are highly resistant to if not immune from fire blight.

SUMMARY

Fifty-six species, representing eleven genera of plants, were inoculated with the fire-blight organism, *Bacillus amylovorus*. Some evidence of the degree of susceptibility of plants in certain of these species is presented.

Among the species of Cotoneaster, *C. Dammeri radicans*[6]*, *C. horizontalis**, *C. pannosa**, and *C. salicifolia floccosa** are distinctly susceptible. *Cotoneaster adpressa**, *C. microphylla** and *C. prostrata** may be infected but exhibit a rather marked resistance, while *C. acuminata*, *C. Dielsiana elegans*, *C. Francheti*, *C. lactea*, and *C. Simonsii* have remained unaffected through these experiments. Some plants of *C. frigida** and *C. Harroviana** are distinctly susceptible, while others are not. This appears to be due to variation within the species.

*Pyracantha angustifolia**, *P. crenulata**, *P. crenulata kansuensis**, *P. crenulata Rogersiana**, *P. Gibbsii yunnanensis**, and *P. formosiana** are at least moderately susceptible, while *P. coccinea* and *P. coccinea Lalandii** are somewhat less so.

Symptoms were produced by inoculations upon plants of *Prunus allegheniensis**, *P. Armeniaca*, *P. Besseyi**, *P. cerasifera**, *P. dasycarpa**, and *P. Simonii**, but all of these species appear to be relatively resistant.

Evidence is presented to show that *Chaenomeles sinensis** may be affected by fire blight in nature.

Successful infection experiments were made upon plants of *Diospyros Lotus**, *Heteromeles arbutifolia*, *Pyrus Malus Niedzwetzkyana**, *P. micromalus**, *P. prunifolia**, and *Raphiolepis indica**.

[6] The species and varieties for which no experimental proof of susceptibility has been found in the literature are marked by an asterisk.

The range of those plants that may be affected by fire blight is shown to be in need of further intensive and extensive study.

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RYE INFECTED WITH BUNT OF WHEAT

E. N. Bressman

During the past season two varieties of rye were inoculated with bunt, *Tilletia tritici* (Bjerk.) Winter and *T. levis* Kühn, from wheat and infection was obtained. In 1923 Gaines and Stevenson[1] found bunt on rye at Pullman, Washington. *T. secalis* (Cda.) Kühn has long been known in Europe, according to Kühn.[2]

Other workers have reported bunt on rye. Kirby,[3] in regard to *Tilletia tritici* on rye, says: "The stinking smut of rye has been reported but once in New York. Rye is very resistant to it, and never more than a few infected heads have been found in a field. The stinking smut in wheat commonly found in this state, is caused by another species of smut fungus. Whether this fungus ever attacks rye is unknown."

In 1928 Johnston[4] stated: "In Kansas several thousand heads of rye grown from seed heavily inoculated with bunt were examined and three heads were found which were bunted. The rye was a mixture in badly smutted wheat seed and plants grown from that seed were heavily smutted."

In the last 3 years the writer has received 100 collections of bunt from various American and foreign sources. Sixteen of these collections were placed on spring rye sown at Corvallis in the fall of 1928. None of these infected the spring rye. In the fall of 1929, 26 of the outstanding collections of bunt obtained from the variety Hybrid 128 grown at Corvallis were placed on Oregon Rye Sel. and 5 collections on pure Rosen rye obtained from the Michigan Agricultural College.

The seed was heavily coated with bunt spores from the various collections and sown in rod rows in the cereal nursery at Corvallis. One collection of *Tilletia tritici,* classified as physiologic form 9, in work to be published later, was the only one that infected rye at Corvallis. Six heads of bunt, or 6.1 per cent, were found in 98 heads of Oregon Sel. 1, and 4 heads of bunt, or 3.5 per cent, were found in 115 heads of Rosen rye. None was found in either the checks or rows inoculated with other forms of bunt. There were about 4,000 heads of rye in these trials.

[1] Gaines, E. F., and F. J. Stevenson. Occurrence of bunt in rye. Phytopath. 13: 210–215. 1923.

[2] Kühn, J. *Tilletia secalis,* eine Kornbrandform des Roggens. Bot. Ztg. 34: 470–471. 1876.

[3] Kirby, R. S. Diseases of small grains. Cornell Univ. Col. Agr. Ext. Bul. 157: 66. 1927.

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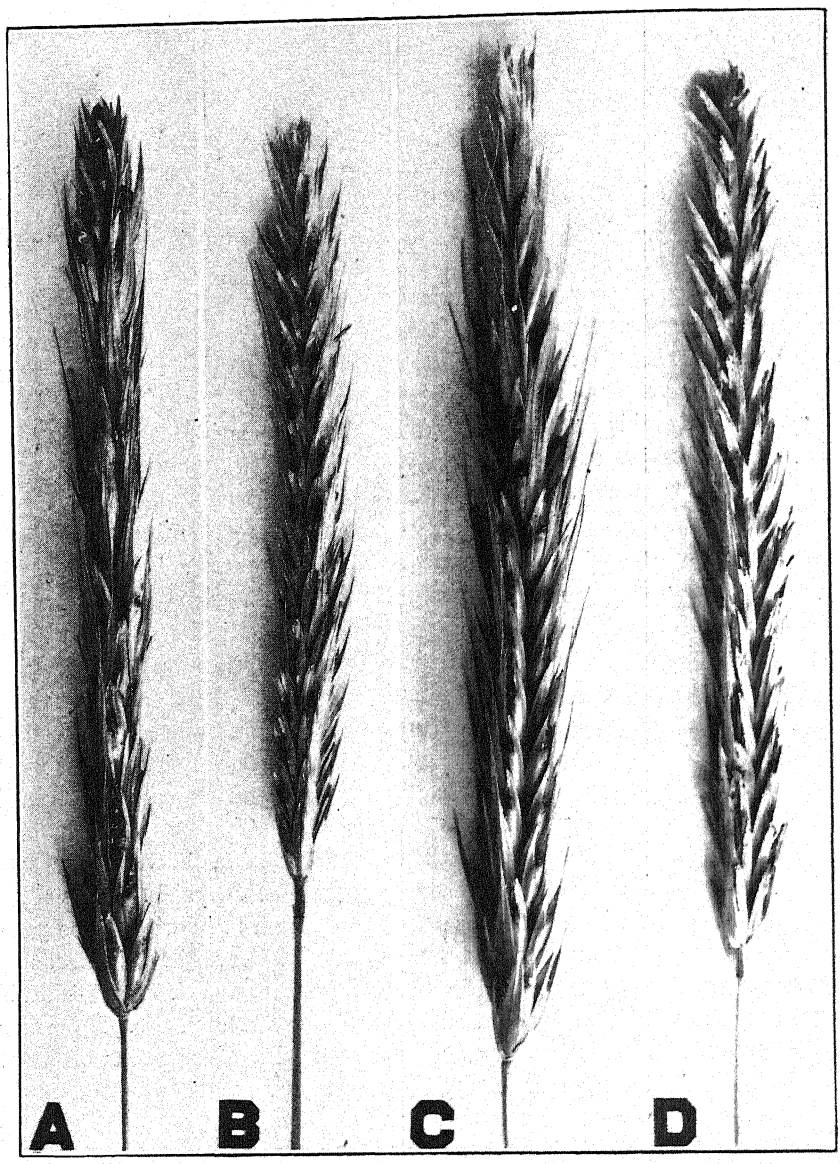


FIG. 1. Normal and smutted heads of rye. A. Normal Oreg. Sel. 1 (note sterility). B. Smutted Oreg. Sel. 1. C. Normal Rosen. D. Smutted Rosen. Corvallis, Oreg. 1930.

The collection of bunt that infected rye was obtained from Morrow County, Oregon, an important wheat-growing section. It is one of the most virulent strains on wheat obtained by the writer and infects many of the so-called resistant varieties.

In duplicate material from Pendleton, Oregon, grown and counted for the writer by J. Foster Martin, 133 heads of bunted rye were found in the 2 varieties. Oregon Rye Sel. No. 1 was inoculated with 15 collections of bunt and Rosen rye with 5 collections in the Pendleton trials of which there were about 6,000 heads. Both species of bunt produced infection. Al-

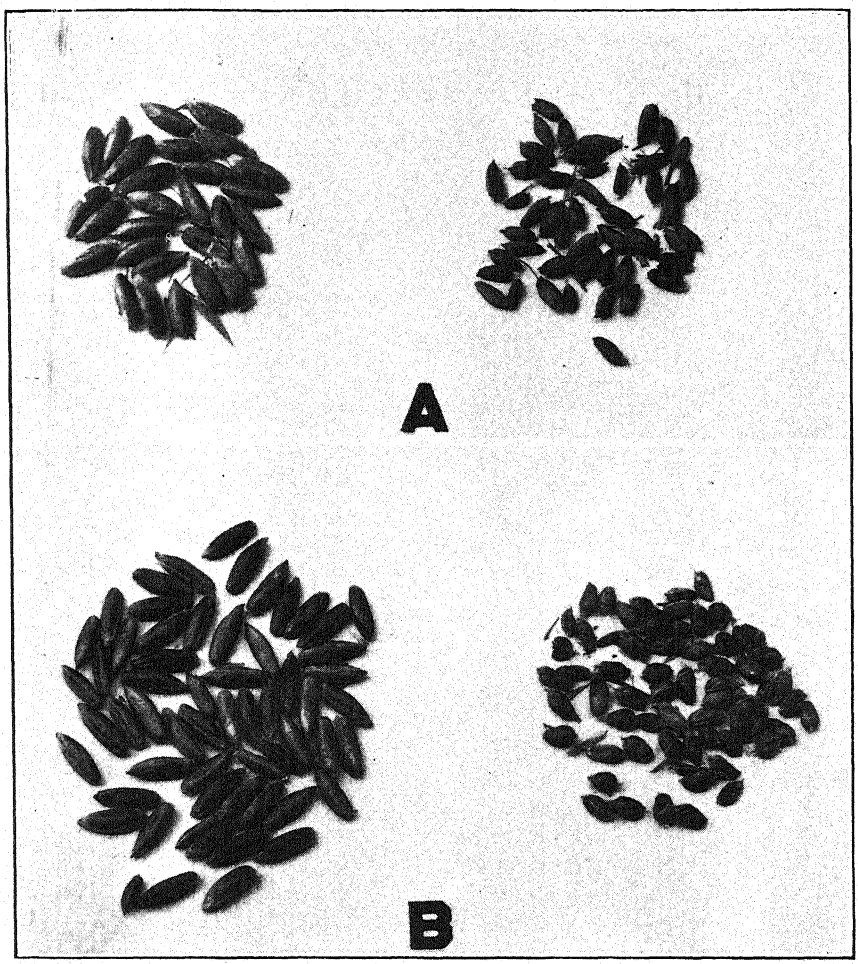


FIG. 2. Normal and smutted kernels of rye. A. Normal kernels and bunt balls of Oreg. Sel. 1. B. Normal kernels and bunt balls of Rosen. Corvallis, Oreg. 1930.

though several collections of bunt infected rye at Pendleton, physiologic form 9 gave the highest percentage.

Figure 1 shows normal and bunted heads of both varieties. It shows no evidence of dwarfing of the bunted heads of these 2 varieties.

The number of bunt balls found in the diseased heads of the Oregon rye is of interest. There is, ordinarily, considerable sterility in this variety and many of the flowers do not produce seed. The diseased heads produced bunt balls in practically all florets. Figure 2 shows typical normal kernels and bunt balls from the 2 varieties and gives an idea of the number of bunt balls formed in comparison with the number of grains in a typical head.

The bunt produced on rye at Corvallis had the typical odor of wheat bunt. The spores averaged about 19 microns in diameter and were typical of *Tilletia tritici*. This material is being used in additional trials to determine definitely whether or not *T. secalis* is a form of *T. tritici* and should be designated as such.

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PHARMACIEN FRECHOU AND THE GERMINATION OF SCLEROSPORA OOSPORES

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It was in 1878 and 1879 that *Sclerospora graminicola* (Sacc.) Schroet. was first reported from Italy, from France, and from Germany, the first accounts of its morphology and development were presented, and its status as the type species of a new genus was established superseding tentative affiliations with Peronospora or Protomyces. The work done by the first investigators of this fungus, chiefly Saccardo, Passerini, and Schroeter is familiar to all. These pioneers did not secure germination of the resting spores even though Schroeter, realizing the importance of working out this phase of the life history, attempted it repeatedly. In the activity which followed calling attention to this fungus other investigators persevered in the endeavor to fill in the gaps in its life history and in 1884 Prillieux reported M. Frechou's success in germinating the oospores.

Since these early years of investigation this fungus has been found on various grasses (chiefly Setarias), both cultivated and wild, in many parts of the world and many of the problems which it involves have been studied by mycologists and plant pathologists. The resting spores, consisting of the single oospore closely enveloped in the thickened, golden to chestnut colored oogonial wall, have been subjected to morphologic and cytologic study, have been used as inoculum in infection tests, and on good evidence have been assumed to be the means by which the fungus survives unfavorable conditions, persists from season to season, and spreads from country to country.

Yet, during almost half a century of such investigation, no further cases of oospore germination were reported and nothing was added to the meagre knowledge of this phase of the life history.

Now, however, within the past year or two, in a recrudescence of activity in the study of this downy mildew, Hiura[1] not only has reported his own successful germination of resting spores of the fungus from cultivated millet in Japan and, presumably from the same host, in the United States, but also has mentioned seeing these bodies successfully germinated by DaNami; while, recently, Evans and Harrar,[2] working under Melhus, report having repeated the procedure successfully with oospores from

[1] Hiura, M. A simple method for the germination of oospores of Sclerospora graminicola. Science, n. s. 72: 95. 1930.

[2] Evans, M. M., and George Harrar. Germination of the oospores of Sclerospora graminicola (Sacc.) Phytopath. 20: 993–998. 1930.

foxtail grass, *Setaria viridis,* at Ames, Iowa, and give the first published drawings of the process.

These papers, unfortunately, do not clear up the confusion that exists among various references in mycological literature to the previous germination of *Sclerospora graminicola* oospores. Gäumann,[3] for example, in his useful and authoritative "Vergleichende Morphologie der Pilze," p. 81, after the statement that germination of the oospores takes place "bei Sclerospora und Peronospora durch ein Keimschlauch, der in der Wirtspflanze zu einem Mycel auswächst" gives deBary 1865 as authority but it is to be feared, without adequate grounds, while Dodge in his extensively revised translation hardly improves the situation by adroitly omitting reference to deBary, thus leaving the statement unsupported. Fitzpatrick in his accurate and comprehensive account of the "Phycomycetes" refers to the method of oospore germination as undetermined. Hiura refers to Magnus as the investigator who succeeded previously in germinating the oospores, while Evans and Harrar point out that there have been confusions and uncertainties and errors in such references without helping to clear up the situation.

In the abundant literature on *Sclerospora graminicola,* from the time of its discovery until the recent work just noted, there is, as far as the writer, is aware, but one reference to any observation of the germination of these resting spores. This, as noted above, is contained in a contribution read by Prillieux[4] before the Societé Botanique de France, séance of December 14, 1884, and published in the Bullétin of that Society. In this, M. Prillieux reports that M. Frechou, pharmacist in the village of Nerac in the Midi of France, in the preceding year had called to his attention *Peronospora setariae* (*Sclerospora graminicola*) on *Setaria verticillata,* and from his own observations and those of M. Frechou, he describes accurately the season and nature of development of both the conidial and oosporic phase of the fungus. He then continues, "Les oöspores, ou spores d'hiver, apparaissent vers le mois de septembre, en grand nombre, à l'intérieur des feuilles; elles sont globuleuses, jaunâtres, ont une paroi épaisse et lisse, et sont contenues dans des oögones ovales ou globuleux à paroi mince. Ces spores dormantes germent au printemps suivant. M. Frechou les a vues émettre un tube comme celles du *Peronospora viticola,* mais n'a pu poursuivre au delà l'étude de leur developpement. Il est donc encore incertain si l'oöspore du *Peronospora setariae* peut produire directement un stipe conidiophore, comme je l'ai annoncé pour le Peronospora de la Vigne."

[3] Gäumann, E. Vergleichende Morphologie der Pilze. Gustav Fischer. Jena. 1926.

[4] Prillieux, E. Sur le Peronospora setariae. Bul. Soc. Bot. France. 31: 397–398. 1884.

This descriptive note, even though scanty and without any supporting illustrations, none the less carries a conviction that germination of the oospores actually was witnessed and it apparently is the basis of the general impression that the oospores germinate by germ tubes in a manner somewhat resembling that of Peronospora or of the grapevine mildew.

It is rather surprising that this lone account of oospore germination in this fungus should have escaped attention so completely. To be sure, the writer,[5] thinking that it might be of interest to others working in this field, referred to it in a discussion of "Production and Dispersal of Conidia in the Philippine Sclerosporas of Maize" in 1921 (p. 273), but even so it remained unnoted either because readers were overcome with fatigue before they had reached this point in that somewhat lengthy paper or because in that sentence the word "paramacien," one of the five unscotched typographical errors in the paper, so intrigued them with its possible protozoan connotations that the potentialities of the reference itself escaped them.

Yet this neglected report of 1884 still has certain mycologic and historic interest. As far as we know, it is not only the first description of the method of oospore germination in *Sclerospora graminicola,* but also the only description of this process during nearly fifty years' investigation of this and other species of the genus. Moreover, in revealing this tantalizing glimpse of Monsieur Frechou, Prillieux's report possesses a certain human interest. Ce pharmacien, que diable allait-il faire dans cette galère? Who was this obscure investigator whose significant observation transmitted unostentatiously through Prillieux has been credited to famous mycologists such as deBary and Magnus? One pictures him, a quiet man with an observing eye and seeking mind, on whose time the duties of compounding a few simple prescriptions for the village made no excessive demands, retiring to the corner of his orderly and aromatic little shop to look through his treasured microscope at diatoms as well as drugs, at moth-wing scales and mildews, at pollen grains, and Peronospora spores. One imagines him in the cool of the evening sitting in his garden and over a bottle of wine discussing with his friend Prillieux questions and problems in the science dear to them both. Pharmacien Frechou, the first to work out the germination of Sclerospora oospores, an accomplishment which it has taken mycologists fifty years to repeat,—what other interesting observations did he make that have, alas, been lost to us? Any further knowledge of him and of his work would, indeed, be welcome.

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[5] Weston, Wm. H., Jr. Production and dispersal of conidia in the Philippine Sclerosporas of Maize. Jour. Agr. Res. 23: 239–278. 1923.

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STUDIES OF THE FUNGICIDAL ACTION OF CERTAIN DUSTS AND SPRAYS IN THE CONTROL OF APPLE SCAB[1]

J. M. HAMILTON

INTRODUCTION

Investigations of the effectiveness of fungicides in the control of diseases of orchard fruits have consisted chiefly of field spraying and dusting work and laboratory studies. While these types of experimentation have yielded very valuable results, each has certain important limitations. In orchard work the environment must be accepted without control and the complexity of variables frequently prevents adequate analysis of results. Furthermore, years of experimentation may elapse before conditions suitable for certain phases of the work are encountered. In laboratory experiments, on the other hand, certain factors of environment may be very accurately controlled. However, the conditions are likely to be so different from those encountered in nature that an interpretation of results obtained from laboratory work can be applied to field conditions only with due reservations. It has seemed desirable in work with apple scab to supplement the field and laboratory studies with greenhouse experiments, in which the fungicides could be applied at will to plants on which the disease was induced by inoculation under partly controlled conditions. This work was begun by Keitt and Jones (29),[2] who developed the technique and conducted preliminary studies. The writer has continued this line of work with special attention to a study of the comparative efficiency of various sulphur fungicides and the relation of certain factors of the environment to their effectiveness.

No attempt will be made to review the extensive literature relative to the fungicidal action of sulphur and its compounds, as this seems to have been sufficiently accomplished in the papers of Windisch (60), Barker,

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

Grateful acknowledgments are made to Dr. G. W. Keitt, at whose suggestion these studies were undertaken, for his kind suggestions and criticisms throughout the investigation and preparation of the manuscript.

[2] Reference is made by number (italic) to "Literature Cited," p. 520.

Gimingham, and Wiltshire (2), Doran (10), Young (61), Vogt (54), Barker (3, 4), Roach and Glynne (44), Young and Williams (62), Goldsworthy (17), Goodwin and Martin (24, 25), Williams and Young (57, 58), Marsh (34), Martin (36), Roach (45), Goodwin, Martin, and Salmon (21) and others. In view of the great number of contributions on the specific use of sulphur for apple-scab control and the limitations encountered in an interpretation of much of the data, a general review of this literature in the present paper appears to be unwarranted. Studies of the epidemiology and control of apple scab and of the ascigerous stage of *Venturia inaequalis* Cke. and Wint. are reported in papers of Keitt and Jones (29) and Wilson (59) and in literature cited by them. As seems necessary, references will be made to the more pertinent work in the appropriate connections in the body of the paper.

GREENHOUSE AND FIELD STUDIES—MATERIALS[3]

The materials used are listed and briefly described as follows:

Sulphur Dusts

Super-sulfodust. A proprietary dusting sulphur prepared by the Niagara Sprayer and Chemical Co., Inc. It is guaranteed to contain not less than 92 per cent of pure sulphur and not more than 8 per cent of inert ingredients. The sulphur has a fineness to permit 98 per cent to pass through a 300-mesh screen.

Sulphur-arsenate dust. Prepared by thoroughly mixing 10 parts by weight of powdered arsenate of lead with 90 parts of Super-sulfodust.

Kolodust. A proprietary Bentonite-sulphur dust prepared by the Niagara Sprayer and Chemical Co., Inc., guaranteed to contain not less than 90 per cent of sulphur and not more than 10 per cent of inert ingredients. It is a mixture of Bentonite-sulphur (colloidal) with finely ground dusting sulphur which has a fineness to permit 98 per cent to pass through a 300-mesh screen.

Kolotex. A proprietary Bentonite-sulphur dust containing arsenate of lead prepared by the Niagara Sprayer and Chemical Co., Inc.,

[3] Acknowledments are made to the following companies for the preparation and donation of materials used in this investigation:
Niagara Sprayer and Chemical Co., Inc., Middleport, New York.
Stauffer Chemical Company of Texas, Houston, Texas.
Koppers Company Laboratories, Mellon Institute, Pittsburgh, Pennsylvania.
Pacific Gas and Electric Company, San Francisco, California.
Cream City Chemical Works, Milwaukee, Wisconsin.
Standard Oil Company of Indiana, Whiting, Indiana.
Bayer-Semesan Company, Inc., Wilmington, Delaware.
Corona Chemical Division, Pittsburgh Plate Glass Co., Milwaukee, Wisconsin.

guaranteed to contain not less than 77 per cent of sulphur and not less than 10 per cent of arsenate of lead. The sulphur is a mixture of Bentonite-sulphur (colloidal) with finely ground dusting sulphur which has a fineness to permit 98 per cent to pass through a 300-mesh screen. The arsenate of lead was guaranteed to contain not less than 1.95 per cent of its own weight of total arsenic (as metallic) and not over 0.5 per cent of total arsenic in water-soluble forms (as metallic).

Bentonite-sulphur. Prepared by the Niagara Sprayer and Chemical Co., Inc. Bentonite-sulphur is the finely divided (colloidal) superactive ingredient of the Niagara Kolo Dusts. It is prepared by fusing sulphur into bentonite, with which it has been previously blended.

Pomodust. A proprietary 90–10 sulphur-arsenate dusting mixture prepared by the Niagara Sprayer and Chemical Co., Inc., guaranteed to contain not less than 87 per cent of pure sulphur and not less than 10 per cent of arsenate of lead. The sulphur has a fineness to permit 98 per cent to pass through a 300-mesh screen. The arsenate of lead was guaranteed to contain not less than 1.95 per cent of its own weight of total arsenic (as metallic) and not over 0.5 per cent of the total arsenic in water-soluble forms (as metallic).

Sublimed sulphur. Anchor Brand Velvet Flowers of Sulphur, the sulphur component of "oxidized sulphur" prepared by the Stauffer Chemical Company of Texas. It has a Chancel test of 76.2° C. It runs 95 to 99 per cent through a 200-mesh screen and is 100 per cent pure.

Oxidized sulphur. A proprietary "oxidizing sulphur," prepared by the Stauffer Chemical Company of Texas. This dusting mixture was composed of sublimed sulphur, 96 per cent; potassium permanganate, 2 per cent; and a catalyst, 2 per cent.

Ferrox sulphur dust. A ground sulphur dust with size of particle averaging 5 microns, prepared by the Koppers Company Laboratories from Ferrox flotation sulphur, a by-product in the purification of manufactured gas.

Wettable Sulphur Preparations[4]

Ferrox flotation sulphur. Wettable sulphur paste with size of particles averaging 3 microns, a by-product in the purification of manufactured gas, containing from 2 to 6 per cent iron oxide in a very finely divided state. Prepared by the Koppers Company Laboratories.

[4] The concentration of the washed and unwashed flotation sulphur sprays used in these experiments was calculated on dry-sulphur basis. These sulphurs do not differ from one another in so far as characteristics of the sulphur particle are concerned.

Thylox flotation sulphur (unwashed). Wettable sulphur paste with size of particles averaging 3 microns, a by-product in the purification of manufactured gas from which all the impurities (mainly small percentages of sodium thiosulphate and sodium thiocyanate) have not been washed. Prepared by the Koppers Company Laboratories.

Gray flotation sulphur. Wettable sulphur paste with size of particles averaging 3 microns, a by-product in the purification of manufactured gas containing nickel. Prepared by the Koppers Company Laboratories.

P. G. & E. sulphur paste. Wettable sulphur paste known as black gashouse sulphur in California and of the same type as Gray flotation sulphur. Prepared by the Pacific Gas and Electric Company.

Koloform. A proprietary wettable Bentonite-sulphur dust prepared by the Niagara Sprayer and Chemical Co., Inc., guaranteed to contain 54 per cent of sulphur and not more than 46 per cent of inert ingredients.

Sprays Containing Lime-sulphur

Lime-sulphur (liquid). A commercial product prepared by Cream City Chemical Works. The specific gravity varied little from 1.8602.

Lime-sulphur, aluminum sulphate mixture (Kelsall's spray). Aluminum sulphate, 3½ pounds to 40 gallons; lime-sulphur, 1–40; and calcium arsenate, ¾ pound to 40 gallons. (Brittain, Kelsall, and Hockey (6).)

Arsenical Compounds

Arsenate of lead. A commercial powdered acid arsenate of lead, prepared by the Niagara Sprayer and Chemical Co., Inc., guaranteed to contain not less than 98 per cent of lead arsenate, not less than 30 per cent of total arsenic oxide (As_2O_5), and not over 0.5 per cent of the total arsenic in water-soluble forms (as metallic).

Calcium arsenate. A commercial powdered calcium arsenate product, prepared by the Niagara Sprayer and Chemical Co., Inc., guaranteed to contain not less than 70 per cent of tri-calcium arsenate, not less than 40 per cent of total arsenic oxide (As_2O_5), and not over 0.5 per cent of the total arsenic in water-soluble forms (as metallic).

Tri-lead arsenate. A basic lead arsenic product, made by the Sherwin-Williams Company, Cleveland, Ohio, guaranteed to contain not less than 25 per cent arsenic oxide and not over 1 per cent of the total arsenic in water-soluble forms (as metallic).

Copper Fungicides

Bordeaux mixture 4–4–50. Chemically pure hydrated lime and copper sulphate were used. No correction was made for use of hydrated instead of stone lime in the composition of the spray.

Copper-lime-arsenate dust. A mixture composed of 10 per cent by weight of finely ground anhydrous cupric sulphate, 80 per cent of hydrated lime, and 10 per cent of powdered arsenate of lead.

Emulsified Oils

L20. A white oil emulsion of the inert type. Prepared by the Standard Oil Company of Indiana.

L202. A white oil emulsion with a lower degree of stability and a higher percentage of oil than held by L20. Prepared by the Standard Oil Company of Indiana.

L205. A white oil emulsion, L20, containing a form of sulphur. Prepared by the Standard Oil Company of Indiana.

Mercurial Compounds

K–1–CB. A proprietary organic ethyl mercury chloride compound with water-soluble and volatile toxic material. Prepared by the Bayer-Semesan Company, Inc.

K–1–GB. A proprietary organic ethyl mercury chloride compound with water-soluble and comparatively nonvolatile toxic material. Prepared by the Bayer-Semesan Company, Inc.

117. A proprietary organic hexamethylene bichloride of mercury compound on a talc base. Prepared by the Corona Chemical Division, Pittsburgh Plate Glass Company.

117C. A proprietary organic hexamethylene bichloride of mercury compound on a bentonite-mixture base. Prepared by the Corona Chemical Division, Pittsburgh Plate Glass Company.

117E. A proprietary organic hexamethylene bichloride of mercury compound on a bentonite-mixture base. The material is acid and contains free mercuric chloride. Prepared by the Corona Chemical Division, Pittsburgh Plate Glass Company.

118. A pure monobasic mercury compound without a base. Prepared by the Corona Chemical Division, Pittsburgh Plate Glass Company.

Other Materials

Calcium monosulphide U.S.P. Contained approximately 68 per cent calcium sulphide.

Casein-lime. "Kayso," a proprietary product obtained from the Griffiths Milk Products Company, Chicago, Illinois.

Lime. The hydrated and stone lime were C.P. products. The hydrated lime was used unless otherwise stated. The stone lime contained 98 per cent calcium oxide.

Soft soaps. Twentieth Century Soap and XXX Cork Tile Soap, made by Theo. B. Robertson Products Co., Inc., Chicago, Illinois. The 20th Century Soap is a neutral sodium soft soap containing 0.5 per cent alkaline salts but no free caustic alkali. The XXX Cork Tile Soap is a neutral potassium soft soap containing 0.12 per cent alkaline salts but no free caustic alkaline.

Other chemicals. The chemicals used other than the above were C.P. products. Distilled water was used in the preparation of all the fungicidal treatments made in the greenhouse unless otherwise stated.

APPARATUS AND METHOD

The moist chamber (Fig. 1) devised by Keitt and Jones (29) was used to accomplish spore germination and infection at the desired temperatures. Two potted Wealthy apple trees (one to three years old) with an average of two to three twigs each (Fig. 2) were used for each experiment. In all cases, unless otherwise stated, the trees were naturally infected by means of ascosporic inoculum of *Venturia inaequalis*. Overwintered apple leaves, bearing abundant perithecia which had been brought to maturity under suitable conditions of temperature and moisture, were placed on wire-net trays above the experimental trees. Ascospores were discharged, and their abundance was observed by microscopic examination of Petri plates exposed in representative situations in the chamber. Fungicidal treatments were made at suitable intervals before or after inoculation by means of a 2½-gallon "Hudson Sprayer" or a small garden hand duster. All moist treatments supplementary to those given during the period of inoculation were provided by placing the plants in the moist chamber at the same temperature used during the inoculation period. After removal from the moist chamber the trees were placed in the greenhouse, unless otherwise stated. After an adequate incubation period for the disease to develop, records were made of the number of lesions on each infected leaf per twig. The maximum number of lesions recorded on any one leaf was 100, regardless of any greater amount of infection, as counts could not be made with satisfactory accuracy if more than 100 lesions were present and a quantitative record above 100 would add little value to the data. Only the infection on the upper surface of the leaves was considered.

EXPERIMENTS IN WHICH THE FUNGICIDES WERE APPLIED BEFORE INFECTION

The widely accepted theory that underlies the application of most fungicides to plants in foliage is that effective treatments must precede

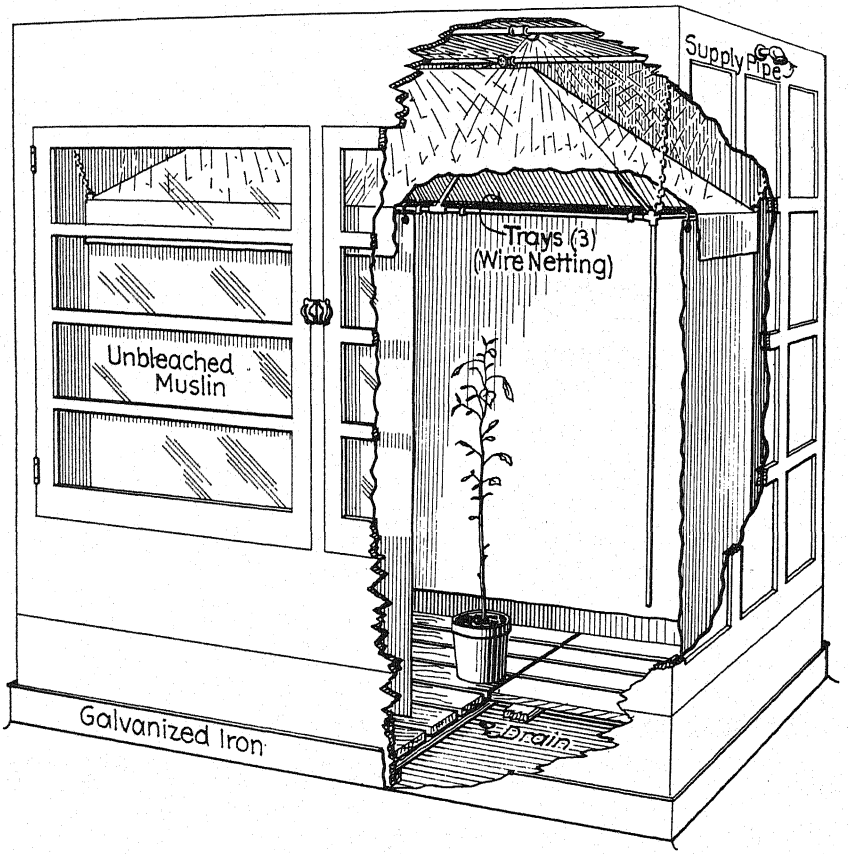


FIG. 1. Inoculation chamber. The temperature in the inner chamber is regulated by the temperature of the water sprayed upon it. The desired temperature is achieved by mixing hot and cold water from supplies kept at suitable constant temperatures. A uniform saturated humidity is assured by wetting the inside of the inner chamber before using. The cloth parts of the inner chamber are washed and sterilized at frequent intervals to kill microorganisms which attack the cloth and might give off gases which inhibit infection.

and prevent infection. Attention has been focused, therefore, upon some of the phenomena of fungicidal effectiveness when applications were made before the infection period began.

The data reported by Keitt and Jones (29) on the relation of time of application and temperature to the effectiveness of certain fungicides in the control of *Venturia inaequalis* in the greenhouse under controlled conditions show that sulphur treatments gave excellent control when the applications were made 24 hours before inoculation, while Bordeaux mixture gave almost no control under the same conditions. Greaney (26), in an

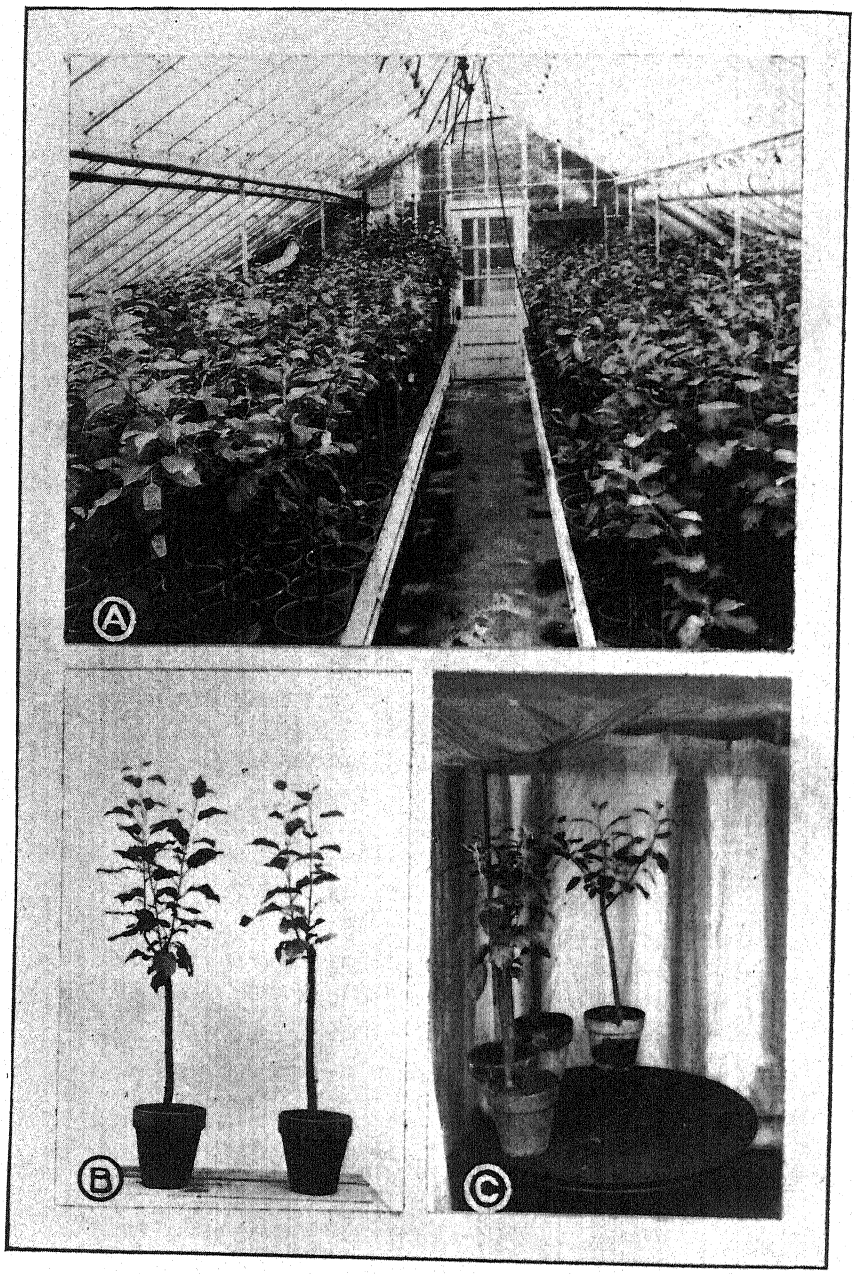


FIG. 2. A. Section of greenhouse showing potted plants used in experimental work. B. Typical potted Wealthy apple trees used in inoculation experiments. C. Turntable used to obtain uniformity of treatment of trees in inoculation and washing experiments.

TABLE 1.—*Relations of certain fungicidal applications to infection of Wealthy apple leaves by ascospores of Venturia inaequalis*

Fungicidal treatment		Inoc. chamber		Final results averaged per twig		
Series, date, and fungicide[a]	Time applied	Temp.	Period in	Leaves infected	Max. lesions on one leaf	
		°C.	Hrs.	No.	No.	Rel. No.
Series 1, 4–20–26						
Untreated		6	32	1.7	8.5	100
L-S 1–40 + AL 1–50	24 hrs. before inoc. began[b]	6	32	0.0	0.0	0
S-Ars. dust 90–10	"	6	32	0.0	0.0	0
Series 2, 4–24–26						
Untreated		7	42	2.7	13.5	100
L-S 1–40 + AL 1–50	24 hrs. before inoc. began[b]	7	42	0.0	0.0	0
S-Ars. dust 90–10	"	7	42	0.0	0.0	0
Series 3, 4–27–26						
Untreated		7	32	1.7	7.0	100
L-S 1–40 + AL 1–50	24 hrs. before inoc. began[b]	7	32	0.0	0.0	0
S-Ars. dust 90–10	"	7	32	0.0	0.0	0
Series 4, 5–7–26						
Untreated		10	27	3.3	40.0	100
L-S 1–40 + AL 1–50	24 hrs. before inoc. began	10	27	0.0	0.0	0
S-Ars. dust 90–10	"	10	27	0.0	0.0	0
Series 5, 5–11–26						
Untreated		10	27	4.0	16.5	100
L-S 1–40 + AL 1–50	24 hrs. before inoc. began	10	27	0.0	0.0	0
S-Ars. dust 90–10	"	10	27	0.0	0.0	0
Series 6, 5–28–26						
Untreated		20	24	2.8	19.1	100
L-S 1–40 + AL 1–50	12 hrs. after inoc. began	20	12	0.0	0.0	0
S-Ars. dust 90–10	"	20	12	1.0	2.0	11
L-S 1–40 + AL 1–50	24 hrs. after inoc. began	20	24	0.0	0.0	0
S-Ars. dust 90–10	"	20	24	3.0	14.6	76

TABLE 1.—(*Continued*)

Fungicidal treatment		Inoc. chamber		Final results averaged per twig		
Series, date, and fungicide[a]	Time applied	Temp.	Period in	Leaves infected	Max. lesions on one leaf	
		°C.	Hrs.	No.	No.	Rel. No.
Series 7, 6–2–26						
Untreated		20	24	2.7	18.7	100
L-S 1–40 + AL 1–50	12 hrs. after inoc. began	20	12	0.0	0.0	0
S-Ars. dust 90–10	" "	20	12	0.0	0.0	0
L-S 1–40 + AL 1–50	24 hrs. after inoc. began	20	24	1.0	1.0	5
S-Ars. dust 90–10	" "	20	24	2.0	13.2	71
Series 8, 4–11–27						
Untreated		6	45	3.0	15.7	100
L-S 1–40 + AL 1–50	24 hrs. before inoc. began[b]	6	45	0.0	0.0	0
S-Ars. dust 90–10	" "	6	45	0.0	0.0	0
L-S 1–40 + AL 1–50	45 hrs. after inoc. began	6	45	0.0	0.0	0
S-Ars. dust 90–10	" "	6	45	2.5	10.5	67
Series 9, 4–14–27						
Untreated		6	45	2.0	7.3	100
L-S 1–40 + AL 1–50	24 hrs. before inoc. began[b]	6	45	0.0	0.0	0
S-Ars. dust 90–10	" "	6	45	0.0	0.0	0
L-S 1–40 + AL 1–50	45 hrs. after inoc. began	6	45	0.0	0.0	0
S-Ars. dust 90–10	" "	6	45	1.0	6.0	82
Series 10, 4–18–27						
Untreated		7	15	1.8	5.0	100
L-S 1–40 + AL 1–50	15 hrs. after inoc. began	7	15	0.0	0.0	0
S-Ars. dust 90–10	" "	7	15	3.0	8.2	164
Untreated		7	20	3.5	31.5	100
L-S 1–40 + AL 1–50	20 hrs. after inoc. began	7	20	0.5	3.5	11
S-Ars. dust 90–10	" "	7	20	3.5	13.2	42
Untreated		7	25	4.5	71.7	100
L-S 1–40 + AL 1–50	25 hrs. after inoc. began	7	25	0.5	5.5	8
S-Ars. dust 90–10	" "	7	25	2.2	14.5	20

TABLE 1.—(*Continued*)

Fungicidal treatment		Inoc. chamber		Final results averaged per twig		
Series, date, and fungicide[a]	Time applied	Temp.	Period in	Leaves infected	Max. lesions on one leaf	
		°C.	Hrs.	No.	No.	Rel. No.
Untreated		7	30	4.2	90.2	100
L-S 1–40 + AL 1–50	30 hrs after inoc. began	7	30	0.0	0.0	0
S-Ars. dust 90–10	"	7	30	3.2	31.5	35
Untreated		7	40	3.4	43.0	100
L-S 1–40 + AL 1–50	40 hrs. after inoc. began	7	40	0.0	0.0	0
S-Ars. dust 90–10	"	7	40	4.2	54.8	127
Series 11,[c] 5–20–27						
L-S 1–40 + AL 1–50	24 hrs. before inoc. began[b]	12	45	0.0	0.0	0
S-Ars. dust 90–10	"	12	45	0.5	0.5	1
Untreated		12	15	5.0	37.8	100
L-S 1–40 + AL 1–50	15 hrs. after inoc. began	12	15	1.0	1.0	3
S-Ars. dust 90–10	"	12	15	2.8	10.4	28
Untreated		12	30	5.0	71.2	100
L-S 1–40 + AL 1–50	30 hrs. after inoc. began	12	30	2.0	8.6	12
S-Ars. dust 90–10	"	12	30	4.0	34.0	48
Untreated		12	45	3.6	72.7	100
L-S 1–40 + AL 1–50	45 hrs. after inoc. began	12	45	2.0	10.5	14
S-Ars. dust 90–10	"	12	45	4.3	62.6	86
Series 12, 5–6–27						
L-S 1–40 + AL 1–50	24 hrs. before inoc. began[b]	10	45	0.0	0.0	0
S-Ars. dust 90–10	"	10	45	0.0	0.0	0
Untreated		10	15	4.0	20.4	100
L-S 1–40 + AL 1–50 (wet)[d]	15 hrs. after inoc. began	10	15	0.0	0.0	0
L-S 1–40 + AL 1–50 (dry)[e]	"	10	15	0.0	0.0	0
S-Ars. dust 90–10 (wet)	"	10	15	0.0	0.0	0
S-Ars. dust 90–10 (dry)	"	10	15	2.7	8.3	41
Untreated		10	30	4.6	33.2	100
L-S 1–40 + AL 1–50 (wet)	30 hrs. after inoc. began	10	30	0.0	0.0	0
L-S 1–40 + AL 1–50 (dry)	"	10	30	2.5	4.0	12

TABLE 1.—(*Continued*)

Fungicidal treatment		Inoc. chamber		Final results averaged per twig		
Series, date, and fungicide[a]	Time applied	Temp.	Period in	Leaves infected	Max. lesions on one leaf	
		°C.	Hrs.	No.	No.	Rel. No.
S-Ars. dust 90–10 (wet)	"	10	30	2.3	12.2	37
S-Ars. dust 90–10 (dry)	"	10	30	3.2	28.2	85
Untreated		10	45	4.0	50.2	100
L-S 1–40 + AL 1–50 (wet)	45 hrs. after inoc. began	10	45	0.0	0.0	0
L-S 1–40 + AL 1–50 (dry)	"	10	45	0.0	0.0	0
S-Ars. dust 90–10 (wet)	"	10	45	3.3	8.7	17
S-Ars. dust 90–10 (dry)	"	10	45	4.3	25.3	50
L-S 1–40 + AL 1–50 (dry)	4 days after inoc. began	10	45	1.0	11.0	22
S-Ars. dust 90–10 (dry)	"	10	45	4.0	37.5	75
L-S 1–40 + AL 1–50 (dry)	8 days after inoc. began	10	45	4.3	33.0	66
S-Ars. dust 90–10 (dry)	"	10	45	4.7	64.6	129
Series 13, 5–1–27						
L-S 1–40 + AL 1–50	24 hrs. before inoc. began[b]	10	45	0.0	0.0	0
S-Ars. dust 90–10	"	10	45	0.0	0.0	0
Untreated		10	15	3.7	22.0	100
L-S 1–40 + AL 1–50 (wet)[d]	15 hrs. after inoc. began	10	15	0.0	0.0	0
L-S 1–40 + AL 1–50 (dry)[e]	"	10	15	1.0	3.7	17
S-Ars. dust 90–10 (wet)	"	10	15	1.8	4.2	19
S-Ars. dust 90–10 (dry)	"	10	15	2.3	9.5	43
Untreated		10	30	4.6	81.4	100
L-S 1–40 + AL 1–50 (wet)	30 hrs. after inoc. began	10	30	0.0	0.0	0
L-S 1–40 + AL 1–50 (dry)	"	10	30	0.5	0.5	1
S-Ars. dust 90–10 (wet)	"	10	30	3.5	14.5	18
S-Ars. dust 90–10 (dry)	"	10	30	3.0	34.5	42
Untreated		10	45	5.0	92.2	100
L-S 1–40 + AL 1–50 (wet)	45 hrs. after inoc. began	10	45	2.3	17.6	19
L-S 1–40 + AL 1–50 (dry)	"	10	45	2.6	9.6	10
S-Ars. dust 90–10 (wet)	"	10	45	3.7	41.2	45
S-Ars. dust 90–10 (dry)	"	10	45	4.8	88.5	96
L-S 1–40 + AL 1–50 (dry)	4 days after inoc. began	10	45	2.3	24.3	26

TABLE 1.—(*Continued*)

Fungicidal treatment		Inoc. chamber		Final results averaged per twig		
Series, date and fungicide[a]	Time applied	Temp.	Period in	Leaves infected	Max. lesions on one leaf	
		°C.	Hrs.	No.	No.	Rel. No.
S-Ars. dust 90–10 (dry)	"	10	45	3.3	37.3	40
L-S 1–40 + AL 1–50 (dry)	8 days after inoc. began	10	45	5.2	53.2	58
S-Ars. dust 90–10 (dry)		10	45	2.8	31.7	34
Series 14, 5–25–27						
L-S 1–40	24 hrs. before inoc. began[b]	14	30	0.0	0.0	0
L-S 1–40 + AL 1–50	"	14	30	0.0	0.0	0
Aerated L-S 1–40	"	14	30	0.0	0.0	0
S-Ars. dust 90–10	"	14	30	0.0	0.0	0
Koloform	"	14	30	0.0	0.0	0
Untreated		14	30	3.6	18.2	100
L-S 1–40 (wet)[d]	30 hrs. after inoc. began	14	30	0.0	0.0	0
L-S 1–40 (dry)[e]	"	14	30	1.0	1.0	6
L-S 1–40 + AL 1–50 (wet)	"	14	30	0.0	0.0	0
L-S 1–40 + AL 1–50 (dry)	"	14	30	1.5	1.5	8
Aerated L-S 1–40 (wet)	"	14	30	3.0	13.3	73
Aerated L-S 1–40 (dry)	"	14	30	3.0	10.8	59
S-Ars. dust 90–10 (wet)	"	14	30	1.0	1.5	8
S-Ars. dust 90–10 (dry)	"	14	30	3.5	20.0	110
Koloform (wet)	"	14	30	3.0	21.3	117
Koloform (dry)	"	14	30	2.7	13.0	71
Series 15,[c] 5–28–27						
L-S 1–40	Before inoc. began	14	30	0.0	0.0	0
L-S 1–40 + AL 1–50	"	14	30	0.0	0.0	0
Aerated L-S 1–40	"	14	30	0.0	0.0	0
S-Ars. dust 90–10	"	14	30	0.0	0.0	0
Koloform	"	14	30	0.0	0.0	0
Untreated		14	30	5.5	53.5	100
L-S 1–40	30 hrs. after inoc. began	14	30	0.0	0.0	0
L-S 1–40 + AL 1–50	"	14	30	0.0	0.0	0

TABLE 1.—(*Continued*)

Fungicidal treatment		Inoc. chamber		Final results averaged per twig		
Series, date and fungicide[a]	Time applied	Temp.	Period in	Leaves infected	Max. lesions on one leaf	
		°C.	Hrs.	No.	No.	Rel. No.
Aerated L-S 1–40	"	14	30	2.5	52.5	98
S-Ars. dust 90–10	"	14	30	3.0	17.5	35
Koloform	"	14	30	4.0	79.0	148
Series 16, 3–3–28						
Untreated		2.5	72	2.3	22.0	100
Kolotex	Just before inoc. began	2.5	72	0.0	0.0	0
Series 17, 5–3–28						
Untreated		20	40	4.8	51.8	100
Kolodust	Just before inoc. began	20	40	0.0	0.0	0
BM 4–4–50	"	20	40	2.0	1.8	3
L20 2%	"	20	40	0.0	0.0	0
L202 2%	"	20	40	0.7	1.5	3
L205 2%	"	20	40	0.0	0.0	0
Series 18, 5–18–28						
Untreated		10	40	4.1	32.0	100
Kolodust	Just before inoc. began	10	40	0.0	0.0	0
BM 4–4–50	"	10	40	1.0	2.3	7
L20 2%	"	10	40	0.0	0.0	0
L202 2%	"	10	40	0.0	0.0	0
L205 2%	"	10	40	0.0	0.0	0

[a] L-S = Commercial liquid lime-sulphur. AL = Commercial powdered arsenate of lead. S-Ars. dust = Sulphur-arsenate dust, 90–10. BM = Bordeaux mixture. L20 and L202 = Emulsified oils. L205 = L20 containing a form of sulphur.

[b] The treated trees were subjected to 12 hours in moist chamber, ending 6 hours before inoculation.

[c] The series was incubated outside after treatment.

[d] The trees were subjected to 15 hours in moist chamber at 10° C. after treatment.

[e] The trees were removed after treatment to Grh 20–25° C.

investigation of the effectiveness of sulphur dusts against some cereal rusts under controlled greenhouse conditions, obtained good control when the treatments were applied 12 days before inoculation, provided the plants were kept dry until inoculated.

Excellent control of apple scab was obtained by the present writer from applications made before inoculation, using various sulphur preparations (aerated lime-sulphur 1–40 included), Bordeaux mixture, and certain oil emulsions (Table 1, Ser. 1–5, 8, 9, 11–18). The sulphur fungicides were effective when applied just before inoculation or 24 hours before inoculation. Certain sulphur fungicides and Bordeaux mixture may protect rapidly growing foliage from infection when applied more than 24 hours before inoculation (Table 7). The noteworthy point is that, under the conditions of this investigation, where applications were made before the infection periods began, the plants were not subjected to loss of protection through rain or washing treatments; all of the materials named gave essentially full protection. It is obvious, therefore, that scab is very easy to control during one period favorable for infection, if the fungicidal agent is not washed off. However, the situation is quite different when experimental conditions are modified (1) to test the comparative effectiveness of materials when applied after infection has started, and (2) to subject the protective materials to washing treatments before the experimental plants are inoculated.

EXPERIMENTS IN WHICH THE FUNGICIDES WERE APPLIED AFTER INFECTION

The most critical time for the control of *Venturia inaequalis* under orchard conditions is during the period of rapid expansion of susceptible host parts early in the season, when environmental factors are likely to be more favorable for infection than later in the year. Early infections provide an early secondary inoculum which, particularly in the case of sepal infection, may result in severe scabbing of the fruit. The rapid expansion of host parts in the preblossom period makes adequate protection very difficult and expensive to maintain. A fungicide which would be effective when applied shortly after infection would offer distinct advantages for scab control in emergencies arising when adequate protection could not be provided before the infection period. An investigation of the effectiveness of sprays or dusts applied after inoculation for the control of *V. inaequalis* was begun by Keitt and Jones (29) in the greenhouse under controlled conditions. This work has been continued by the present writer with special attention to a study of the comparative efficiency of various sulphur fungicides and the relation of temperature and moisture to their effectiveness.

Keitt and Jones (29) found a suggestion of the inhibition of *Venturia inaequalis* under controlled conditions when treatments followed infection

TABLE 2.—*Effectiveness of certain fungicidal treatments applied after infection of Wealthy apple leaves by ascospores of Venturia inaequalis*

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 1, 3–22–28									
Untreated	23	46	0	0	0	3.0	16.0	100	100
Oxidized sulphur	23	46	0	0	0	1.7	7.6	48	41
Sublimed sulphur	23	46	0	0	0	1.5	8.0	50	33
$KMnO_4$ 2%	23	46	0	0	0	1.0	1.0	6	3
Ferrox sulphur dust	23	46	0	0	0	1.7	9.2	58	34
Kolotex	23	46	0	0	0	2.0	6.0	38	24
L202 2%	23	46	0	0	0	1.0	3.3	21	12
L205 2%	23	46	0	0	0	0.0	0.0	0	0
L202 2% + Kolodust 8–50	23	46	0	0	0	0.5	2.0	13	7
BM 4–4–50	23	46	0	0	0	2.0	4.6	29	21
L-S 1–40 + AL 1–50	23	46	0	0	0	1.0	1.0	6	7
Series 2, 4–6–28									
Untreated	10	46	0	0	0	5.4	34.1	100	100
Oxidized sulphur	10	46	0	0	0	3.8	18.2	53	29
Sublimed sulphur	10	46	0	0	0	6.0	18.3	54	58
Kolodust	10	46	0	0	0	3.2	15.0	44	30
Kolotex	10	46	0	0	0	4.4	23.8	70	43
Super-sulfodust	10	46	0	0	0	4.3	25.0	73	58
P. G. & E. sulphur paste 5–50	10	46	0	0	0	4.5	15.6	46	33
Ferrox sulphur dust	10	46	0	0	0	3.4	26.8	79	60
Ferrox sulphur paste 5–50	10	46	0	0	0	5.5	34.7	102	103
Ferrox sulphur paste 5–50 + AL 1–50	10	46	0	0	0	3.8	26.0	76	45
L-S 1–40	10	46	0	0	0	0.0	0.0	0	0
L-S 1–40 + AL 1–50	10	46	0	0	0	0.7	1.3	4	1
AL 1–50	10	46	0	0	0	4.6	16.4	48	38
L205 2%	10	46	0	0	0	1.4	3.6	11	9
BM 4–4–50	10	46	0	0	0	3.0	7.0	21	14

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 3, 4–9–28									
Untreated	10	72	0	0	16	4.2	26.0	100	100
Kolodust	10	72	0	0	16	4.0	33.6	129	110
BM 4–4–50	10	72	0	0	16	3.2	11.0	42	51
AL 1–50	10	72	0	0	16	3.8	12.4	48	54
L202 2%	10	72	0	0	16	4.0	13.2	51	71
L205 2%	10	72	0	0	16	2.0	5.0	19	15
L202 2% + Kolodust 8–50	10	72	0	0	16	1.0	6.0	23	9
L-S 1–40	10	72	0	0	16	1.2	3.7	14	5
Series 4, 4–17–28									
Untreated	10	65	0	24	5	6.2	85.5	100	100
Oxidized sulphur	10	65	0	24	5	5.2	44.0	51	35
Sublimed sulphur	10	65	0	24	5	6.0	56.7	66	58
$KMnO_4$ 2%	10	65	0	24	5	1.0	2.0	2	1
Kolotex	10	65	0	24	5	6.0	44.8	52	42
L202 2%	10	65	0	24	5	5.8	12.2	14	14
L205 2%	10	65	0	24	5	4.2	14.2	17	8
L202 2% + Kolotex 8–50	10	65	0	24	5	3.2	5.2	6	5
L-S 1–40 + AL 1–50	10	65	0	24	5	1.2	2.5	3	1
Series 5, 4–18–28									
Untreated	10	64	0	0	24	5.2	45.4	100	100
Oxidized sulphur	10	64	0	0	24	4.3	54.0	119	98
Kolodust	10	64	0	0	24	4.3	36.0	79	50
Ferrox sulphur dust	10	64	0	0	24	3.3	42.0	93	61
Ferrox sulphur paste 5–50	10	64	0	0	24	3.3	38.0	84	97
P. G. & E. sulphur paste 5–50	10	64	0	0	24	4.0	38.6	85	60
Precipitated sulphur	10	64	0	0	24	3.7	35.0	77	58
C-L-Ars. dust 10–80–10	10	64	0	0	24	4.3	70.3	155	123

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 6, 5–12–28									
Untreated	12	48	0	24	13	3.3	28.3	100	100
Kolotex	12	48	0	24	13	0.3	0.3	1	0
NaOH N/14	12	48	0	24	13	0.7	0.7	2	3
Lime 3–50	12	48	0	24	13	2.3	12.6	45	27
Soft soap 1%[c]	12	48	0	24	13	2.3	20.6	73	41
Series 7, 5–19–28									
Untreated	15	42	30	24	18	5.2	64.5	100	100
L-S 1–40	15	42	30	24	18	1.3	9.6	15	8
L-S 1–40 + AL 1–50	15	42	30	24	18	1.0	7.5	12	6
K-l-CB 2–50	15	42	30	24	18	2.6	12.3	19	24
K-l-GB 2–50	15	42	30	24	18	3.3	26.6	41	60
L-S 1–40	15	42	52	1	18	2.5	11.0	17	15
L-S 1–40 + AL 1–50	15	42	52	1	18	0.5	0.5	1	0
K-l-CB 2–50	15	42	52	1	18	3.5	24.5	38	58
K-l-GB 2–50	15	42	52	1	18	4.5	49.0	76	103
Series 8, 6–2–28									
Untreated	17	48	32	3	12	5.2	86.2	100	100
L-S 1–40	17	48	32	3	12	3.0	42.7	50	33
L-S 1–40 + AL 1–50	17	48	32	3	12	5.0	15.0	17	14
AS + L-S + CA $3\frac{1}{2}$–1–$\frac{3}{4}$–40	17	48	32	3	12	1.5	1.0	1	1
Series 9, 5–24–29									
Untreated	15	29	0	18	21	5.5	78.0	100	100
L-S 1–40	15	29	0	18	21	3.3	14.7	19	16
CaS 10–50	15	29	0	18	21	4.3	8.0	10	12
CaS 10–50 + AL 1–50	15	29	0	18	21	1.3	1.3	2	1
Bentonite-sulphur 5–50 + AL 1–50	15	29	0	18	21	2.7	11.0	14	10

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 10, 3–12–28									
Untreated	5	48	0	0	0	3.0	84.0	100	100
Kolotex	5	48	0	0	0	3.0	78.0	93	63
"	5	48	0	0	15	2.3	48.0	57	48
L-S 1–40 + AL 1–50	5	48	0	0	0	1.7	12.3	15	8
"	5	48	0	0	15	1.0	5.3	6	5
Untreated	5	72	0	0	0	4.0	71.3	100	100
Kolotex	5	72	0	0	0	2.5	80.5	113	113
"	5	72	0	0	15	1.0	30.5	43	20
L-S 1–40 + AL 1–50	5	72	0	0	0	2.5	46.5	65	33
"	5	72	0	0	15	1.7	5.0	7	4
Series 11, 4–7–28									
Untreated	10	45	0	0	0	4.2	35.6	100	100
Super sulfodust	10	45	0	0	0	4.4	36.0	101	124
"	10	45	0	0	20	3.3	37.7	106	77
Kolodust	10	45	0	0	0	4.7	50.3	141	137
"	10	45	0	0	20	5.0	55.6	156	168
Kolotex	10	45	0	0	0	5.0	46.0	129	181
"	10	45	0	0	20	3.0	27.4	77	65
L-S 1–40	10	45	0	0	0	1.3	5.0	14	10
"	10	45	0	0	20	0.0	0.0	0	0
L-S 1–40 + AL 1–50	10	45	0	0	0	0.0	0.0	0	0
"	10	45	0	0	20	0.3	0.3	1	0
AL 1–50	10	45	0	0	0	4.8	58.4	164	91
"	10	45	0	0	20	3.0	21.5	60	46
Series 12, 4–26–28									
Untreated	10	48	0	0	0	4.1	47.5	100	100
Kolodust	10	48	0	0	0	3.5	40.0	84	64

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 12, 4–26–28—Continued									
Kolodust	10	48	0	0	18	3.5	69.5	146	96
NaOH N/10	10	48	0	0	0	2.5	2.5	5	6
"	10	48	0	0	18	2.0	5.8	12	9
Series 13, 3–4–29 (Av. of 2 Ser.)									
Untreated	15	45	0	0	0	5.3	88.8	100	100
"	15	45	0	0	10	5.9	92.8	100	100
Kolodust	15	45	0	0	0	4.9	88.3	100	125
"	15	45	0	0	10	4.0	81.3	89	69
Ferrox sulphur paste 5–50	15	45	0	0	0	3.8	50.0	52	42
"	15	45	0	0	10	2.9	27.3	30	19
Thylox sulphur paste (unwashed) 5–50	15	45	0	0	0	0.8	1.3	2	0
"	15	45	0	0	10	0.0	0.0	0	0
Gray sulphur past 5–50	15	45	0	0	0	4.5	82.5	92	126
"	15	45	0	0	10	4.4	64.7	69	55
Series 14, 3–12–29									
Untreated	15	26	0	0	0	4.5	29.0	100	100
"	15	26	0	0	14	5.0	63.0	100	100
Kolodust	15	26	0	0	0	4.0	23.5	81	69
"	15	26	0	0	14	3.3	48.0	76	56
Ferrox sulphur paste 5–50	15	26	0	0	0	4.0	45.0	155	145
"	15	26	0	0	14	3.0	9.0	14	8
Gray sulphur paste 5–50	15	26	0	0	0	3.0	26.0	90	105
"	15	26	0	0	14	2.3	7.3	12	7
Series 15,[d] 6–17–29									
A Untreated (14 spores to low power)	20	12	0	61	18	5.3	41.0	100	100
B Untreated (1 spore to low power)	20	12	0	61	18	5.5	29.5	100	100

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 15,[d] 6–17–29—Continued									
A Untreated[e]	20	12	0	61	18	5.0	100.0	100	100
A Kolodust	20	12	0	61	18	3.3	30.7	75	49
B Kolodust	20	12	0	61	18	2.7	15.0	51	41
A Kolodust[e]	20	12	0	61	18	0.5	0.5	1	0
A Kolodust[f]	20	12	0	61	18	1.7	1.7	4	2
Series 16, 4–9–29									
Untreated	15	16	0	0	0	4.7	100.0	100	100
"	15	16	0	0	13	4.0	79.7	100	100
Kolodust	15	16	0	0	0	4.0	100.0	100	81
"	15	16	0	0	13	4.0	44.0	55	39
Aerated L-S 1–40	15	16	0	0	0	3.0	50.7	51	37
"	15	16	0	0	13	2.3	35.0	44	31
NaOH N/14	15	16	0	0	0	1.3	3.0	3	1
Soft soap 1%[e]	15	16	0	0	0	1.0	4.7	5	4
"	15	16	0	0	13	0.3	0.7	1	0
Stone lime 3–50	15	16	0	0	0	4.0	100.0	100	66
"	15	16	0	0	13	3.7	24.0	30	29
AL 2–50	15	16	0	0	0	4.0	100.0	100	107
"	16	15	0	0	13	4.7	100.0	125	129
Series 17, 6–12–29									
Untreated	20	35	0	0	0	6.5	100.0	100	100
"	20	35	0	0	12	7.0	100.0	100	100
Bentonite-sulphur 5–50	20	35	0	0	0	6.8	100.0	100	91
Bentonite-sulphur 5–50 + AL 1–50	20	35	0	0	0	6.0	100.0	100	67
"	20	35	0	0	12	4.7	52.0	52	20
CaS 10–50	30	35	0	0	0	5.3	100.0	100	61
CaS 10–50 + AL 1–50	20	35	0	0	0	8.0	100.0	100	126

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 17, 6–12–29—Continued									
CaS 10–50 + AL 1–50	20	35	0	0	12	2.8	32.0	32	12
L-S 1–40	20	35	0	0	0	5.0	79.3	79	44
117 1–50	20	35	0	0	0	5.3	100.0	100	77
"	20	35	0	0	12	6.8	100.0	100	89
117E 1–50	20	35	0	0	0	5.0	52.0	52	20
Series 18, 3–3–29									
Untreated	15	53	29	0	0	4.7	100.0	100	100
CaS 10–50	15	53	29	0	0	4.0	68.3	68	87
"	15	53	29	0	10	4.0	93.5	94	102
L-S 1–40	15	53	29	0	0	4.7	55.3	55	65
"	15	53	29	0	10	4.0	71.5	72	74
L-S 1–40 + AL 1–50	15	53	29	0	0	3.0	51.0	51	47
"	15	53	29	0	10	3.3	34.3	34	41
L-S 1–40 + CA 1–50	15	53	29	0	0	3.3	76.3	76	73
"	15	53	29	0	10	3.7	40.3	40	50
AS + L-S + CA $3\frac{1}{2}$–1–$\frac{3}{4}$–40	15	53	29	0	0	0.0	0.0	0	0
Series 19, 4–6–29									
Untreated	15	48	29	0	0	5.0	100.0	100	100
L-S 1–40	15	48	29	0	0	4.0	67.3	67	44
"	15	48	29	0	8	4.7	80.7	81	65
L-S 1–40 + AL 1–50	15	48	29	0	0	4.3	88.7	89	75
"	15	48	29	0	8	4.3	92.7	93	65
L-S 1–40 + CA 1–50	15	48	29	0	0	5.0	100.0	100	107
"	15	48	29	0	8	4.3	82.5	83	66
AS + L-S + CA $3\frac{1}{2}$–1–$\frac{3}{4}$–40	15	48	29	0	0	3.7	17.7	18	13
"	15	48	29	0	8	2.0	16.0	16	7

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 20, 3–30–29									
Untreated	15	45	30	4	0	7.5	64.0	100	100
L-S 1–40 + AL 1–50 (distilled water)	15	45	30	4	0	4.0	22.3	35	17
"	15	45	30	4	10	2.3	6.0	9	4
L-S 1–40 + AL 1–50 (lake water)	15	45	30	4	0	5.0	21.3	33	18
"	15	45	30	4	10	2.5	5.0	8	3
L-S 1–40 + CA 1–50 (distilled water)	15	45	30	4	0	3.8	23.7	37	20
"	15	45	30	4	10	3.2	4.0	6	4
L-S 1–40 + CA 1–50 (lake water)	15	45	30	4	0	5.3	38.7	60	36
"	15	45	30	4	10	2.5	6.5	10	4
Series 21, 4–5–29									
Untreated	15	48	12	0	0	6.7	100.0	100	100
L-S 1–40 + AL 1–50 (distilled water)	15	48	12	0	0	6.0	63.5	64	38
"	15	48	12	0	12	5.0	55.0	55	18
L-S 1–40 + AL 1–50 (lake water)	15	48	12	0	0	6.5	93.5	94	55
"	15	48	12	0	12	5.5	66.8	67	33
L-S 1–40 + CA 1–50 (distilled water)	15	48	12	0	0	6.2	92.2	92	43
"	15	48	12	0	12	5.5	74.7	75	33
L-S 1–40 + CA 1–50 (lake water)	15	48	12	0	0	6.0	100.0	100	64
"	15	48	12	0	12	5.0	59.5	60	27
Series 22,[d] 5–20–29									
A Untreated (48 spores to low power)	15	46	0	0	0	7.8	100.0	100	100
B Untreated (8 spores to low power)	15	46	0	0	0	7.0	100.0	100	100
A L-S 1–40 + AL 1–50	15	46	0	0	0	4.8	100.0	100	34
B L-S 1–40 + AL 1–50	15	46	0	0	0	4.5	59.0	59	29
A L-S 1–40 + AL 1–50	15	46	0	0	10	3.5	61.0	61	15
B L-S 1–40 + AL 1–50	15	46	0	0	10	3.0	20.0	20	6
A L-S 1–40 + AL 1–50[g]	15	46	0	0	10	3.7	33.7	34	12

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 22,[d] 5–20–29—Continued									
B L-S 1–40 + AL 1–50[g]	15	46	0	0	10	1.5	2.0	2	1
A L-S 1–40 + AL 1–50	15	46	0	0	20	3.3	19.8	20	6
B L-S 1–40 + AL 1–50	15	46	0	0	20	1.8	13.5	14	3
Series 23,[d] 5–28–29									
A Untreated (14.6 spores to low power)	15	32	0	0	0	7.2	100.0	100	100
B Untreated (6.2 spores to low power)	15	32	0	0	0	7.4	89.8	100	100
A L-S 1–40	15	32	0	0	0	4.0	39.6	40	17
B L-S 1–40	15	32	0	0	0	3.4	24.1	27	13
Series 24, 3–21–29									
Untreated	15	48	24	0	12	4.7	36.7	100	100
L-S 1–25	15	48	24	0	12	2.0	9.7	26	15
L-S 1–40	15	48	24	0	12	2.7	7.0	19	13
L-S 1–80	15	48	24	0	12	3.0	17.7	48	33
L-S 1–120	15	48	24	0	12	3.5	19.8	54	38
L-S 1–25 + AL 1–50	15	48	24	0	12	0.7	4.0	11	5
L-S 1–40 + AL 1–50	15	48	24	0	12	2.3	8.3	23	17
L-S 1–80 + AL 1–50	15	48	24	0	12	2.7	3.3	9	8
L-S 1–120 + AL 1–50	15	48	24	0	12	2.0	13.3	36	29
Series 25, 6–9–28									
Untreated	16	34	14	0	8	4.0	40.5	100	100
L-S 1–120	16	34	14	0	8	1.3	7.3	18	16
L-S 1–80	16	34	14	0	8	2.0	5.5	14	20
L-S 1–40	16	34	14	0	8	2.2	2.2	5	4
L-S 1–40 4 hrs.	16	34	14	0	8	1.0	7.0	17	12
L-S 1–40 6.5 hrs.	16	34	14	0	8	0.5	4.0	10	7

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 25, 6–9–28—Continued									
L-S 1–40 8 hrs.	16	34	14	0	8	1.0	1.6	4	3
NaOH N/10	16	34	14	0	8	1.7	3.2	8	8
NaOH N/14	16	34	14	0	8	1.3	2.0	5	4
Series 26, 5–30–28									
Untreated	16	48	0	0	8	5.0	100.0	100	100
L-S 1–40 1 hr.	16	48	0	0	8	5.0	70.7	71	58
L-S 1–40 2 hrs.	16	48	0	0	8	3.3	100.0	100	53
L-S 1–40 4.5 hrs.	16	48	0	0	8	3.8	28.0	28	18
L-S 1–40	16	48	0	0	8	2.2	16.0	16	50
Series 27, 3–29–29									
Untreated	15	45	30	0	0	6.3	100.0	100	100
L-S 1–40 + AL 1–50	15	45	30	0	0	5.3	95.3	95	52
"	15	45	30	0	6	4.7	100.0	100	56
L-S 1–40 + AL 1–50 6 hrs.	15	45	30	0	6	5.0	97.7	98	55
L-S 1–40 + AL 1–50	15	45	30	0	12	4.0	52.7	53	27
L-S 1–40 + AL 1–50 12 hrs.	15	45	30	0	12	4.7	70.3	70	34
L-S 1–40 + AL 1–50	15	45	30	0	18	3.2	28.7	29	14
L-S 1–40 + AL 1–50 18 hrs.	15	45	30	0	18	5.0	68.5	69	33
Series 28, 4–17–28									
Untreated	10	66	0	24	5	4.7	37.0	100	100
L-S 1–40 + AL 1–50	10	66	0	0	0	2.0	4.3	12	8
L-S 1–40 + AL 1–50 (dried with fan)	10	66	0	0	0	1.8	1.6	4	3
L-S 1–40 + AL 1–50	10	66	0	0	4	2.6	5.4	15	10
L-S 1–40 + AL 1–50 + lime 3–50	10	66	0	24	5	1.5	4.5	12	9
L-S 1–25 + AL 1–50	10	66	0	24	5	0.5	1.5	4	3
AS + L-S + CA $3\frac{1}{2}$–1–$\frac{3}{4}$–40	10	66	0	24	5	0.7	1.2	3	2
NaOH N/10	10	66	0	24	5	1.8	4.2	12	7

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 29, 4–10–28									
Untreated	10	72	0	0	0	3.6	54.0	100	100
L-S 1–40 + AL 1–50	10	72	0	0	0	1.0	7.0	13	7
L-S 1–40 + AL 1–50 (dried with fan)	10	72	0	0	0	0.3	0.6	1	1
L-S 1–40 + AL 1–50	10	72	0	0	2	2.2	13.5	25	23
L-S 1–40 + AL 1–50 + Kayso ½–50	10	72	0	0	0	1.2	6.0	11	9
L-S 1–40 + Kayso ½–50 + AL 1–50	10	72	0	0	0	1.0	3.5	6	8
Kayso ½–50 + AL 1–50 + L-S 1–40	10	72	0	0	0	1.0	4.0	7	4
Series 30, 4–24–28									
Untreated	10	48	24	2.5	10	5.5	82.2	100	100
L-S 1–40 + AL 1–50	10	48	24	2.5	10	2.6	10.6	13	10
L-S 1–40 + AL 1–50 + Kayso ½–50	10	48	24	2.5	10	1.5	5.5	7	4
L-S 1–40 + Kayso ½–50 + AL 1–50	10	48	24	2.5	10	2.5	8.5	10	12
Kayso ½–50 + AL 1–50 + L-S 1–40	10	48	24	2.5	10	3.3	22.0	27	21
L-S 1–40 + AL 1–50 + lime 3–50	10	48	24	2.5	10	3.0	26.5	32	16
Series 31, 5–26–28									
Untreated	15	48	42	0	10	5.5	98.8	100	100
L-S 1–40	15	48	42	0	10	4.3	64.3	65	31
L-S 1–40 + AL 1–50	15	48	42	0	10	3.7	70.0	71	28
L-S 1–40 + AL 1–50 + Kayso ½–50	15	48	42	0	10	4.3	100.0	101	50
L-S 1–40 + Kayso ½–50 + AL 1–50	15	48	42	0	10	3.2	31.2	32	11
Kayso ½–50 + AL 1–50 + L-S 1–40	15	48	42	0	10	3.5	73.5	74	37
L-S 1–40 + AL 1–50 + lime 3–50	15	48	42	0	10	4.3	49.6	50	20
Lime 3–50	15	48	42	0	10	4.3	92.0	93	58
Series 32, 4–1–29									
Untreated	15	45	27	5	8	6.0	46.8	100	100
L-S 1–40	15	45	27	5	8	4.0	16.3	35	28

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 32, 4–1–29—Continued									
L-S 1–40 + AL 1–50	15	45	27	5	8	2.3	11.0	24	12
L-S 1–40 + AL 1–50 (basic)	15	45	27	5	8	4.2	13.6	29	18
L-S 1–40 + As_2O_5 .002%	15	45	27	5	8	4.3	21.0	45	37
L-S 1–40 + As_2O_5 .01%	15	45	27	5	8	3.0	15.0	32	16
Series 33, 5–10–29									
Untreated	15	48	0	6	13	7.2	100.0	100	100
L-S 1–40	15	48	0	6	13	3.3	100.0	100	24
L-S 1–40 + AL 1–50	15	48	0	6	13	3.8	34.2	34	8
L-S 1–40 + As_2O_5 .02%	15	48	0	6	13	3.5	91.7	92	27
Series 34, 6–18–29									
Untreated	17	28	0	22	12	4.0	21.0	100	100
L-S 1–40	17	28	0	22	12	4.0	8.3	40	26
L-S 1–40 + AL 1–50	17	28	0	22	12	0.0	0.0	0	0
L-S 1–40 + As_2O_5 .02%	17	28	0	22	12	0.0	0.0	0	0
L-S 1–40 + As_2O_5 .04%	17	28	0	22	12	0.0	0.0	0	0
CaS 10–50	17	28	0	0	0	2.0	11.3	54	42
"	17	28	0	0	10	1.7	5.0	24	15
CaS 10–50 + AL 1–50	17	28	0	0	0	0.5	0.5	2	1
"	17	28	0	0	10	1.0	4.0	19	13
L20 2% + 117 1–50	17	28	0	0	0	2.3	9.0	43	33
L20 2% + 117E 1–50	17	28	0	0	0	2.2	5.2	25	19
Series 35, 6–22–29									
Untreated	21	35	0	69	0	4.0	78.5	100	100
L-S 1–40	21	35	0	69	8	2.5	25.5	32	22
"	21	35	0	45	24	0.0	0.0	0	0
L-S 1–40 + AL 1–50	21	35	0	69	8	0.0	0.0	0	0

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 35, 6–22–29—Continued									
L-S 1–40 + AL 1–50	21	35	0	45	24	1.0	2.3	3	2
L-S 1–40 + As_2O_5 .03%	21	35	0	69	8	0.5	1.0	1	1
"	21	35	0	45	24	0.0	0.0	0	0
As_2O_5 .03%	21	35	0	69	8	4.0	26.0	33	28
"	21	35	0	45	24	2.3	26.7	34	26
Series 36, 4–11–28									
Untreated	10	72	0	0	0	3.8	69.8	100	100
Kolotex	10	72	0	0	0	2.5	26.5	38	30
Kolotex 8–50 + soft soap 1%[c]	10	72	0	0	0	0.7	16.5	24	14
Kolotex 8–50 + Kayso ½–50	10	72	0	0	0	2.8	21.2	30	26
Kolotex 8–50 + lime 3–50	10	72	0	0	0	1.8	5.4	8	6
Kolotex 8–50 + L202 2%	10	72	0	0	0	2.0	12.3	18	9
L-S 1–40 + AL 1–50	10	72	0	0	0	1.4	7.6	11	8
Series 37, 4–29–28									
Untreated	10	48	0	0.5	8	3.7	50.2	100	100
Oxidized sulphur 8–50	10	48	0	0.5	8	4.2	88.0	175	197
Kolodust 8–50	10	48	0	0.5	8	3.6	19.3	38	37
Kolotex 8–50 + soft soap 1%[c]	10	48	0	0.5	8	0.6	1.0	2	2
Kolodust 8–50 + L202 2%	10	48	0	0.5	8	1.0	4.0	8	5
Ferrox sulphur paste 8–50	10	48	0	0.5	8	3.0	24.2	48	56
Series 38, 4–23–29									
Untreated	15	30	0	18	8	7.8	100.0	100	100
L-S 1–40	15	30	0	18	8	3.8	15.8	16	8
L20 2%	15	30	0	18	8	6.7	88.7	89	49
L205 2%	15	30	0	18	8	4.5	20.0	20	9
L20 2% + K-I-CB 2–50	15	30	0	18	8	2.7	8.0	8	3

TABLE 2—(*Continued*)

Series, date, and fungicide[a]	Inoculation chamber		Period in Grh[b] before fung. applied	Subsequent treatment		Final results averaged per twig			
	Temp.	Period in		In Grh	In IC[b]	Leaves infected	Max. lesions on one leaf		Total lesions
	°C.	Hrs.	Hrs.	Hrs.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 38, 4–23–29—Continued									
L20 2% + Bentonite-sulphur 5–50	15	30	0	18	8	5.5	29.8	30	22
Bentonite sulphur 5–50	15	30	0	18	8	6.5	57.0	57	41
Bentonite-sulphur 5–50 + soft soap 1%[b]	15	30	0	18	8	3.2	13.0	13	7
Soft soap 1%[b]	15	30	0	18	8	5.5	57.2	57	37
Series 39, 5–22–29									
Untreated	15	30	0	10	10	6.8	100.0	100	100
L20 2%	15	30	0	10	10	9.0	100.0	100	125
L205 2%	15	30	0	10	10	6.0	100.0	100	89
L20 2% + 117 1–50	15	30	0	10	10	1.7	7.0	7	2
L20 2% + 117c 1–50	15	30	0	10	10	2.0	23.0	23	6
L20 2% + 118 1–50	15	30	0	10	10	3.3	35.3	35	12
L20 2% + K-l-CB 1–50	15	30	0	10	10	4.2	34.8	35	17
Series 40, 5–29–29									
Untreated	15	24	0	0	0	5.5	100.0	100	100
L20 2%	15	24	0	0	0	8.0	100.0	100	87
L205 2%	15	24	0	0	0	5.0	47.5	48	30
L20 2% + 117 1–50	15	24	0	0	0	4.0	16.0	16	10
L20 2% + 117c 1–50	15	24	0	0	0	1.7	11.7	12	5
L20 2% + 118 1–50	15	24	0	0	0	4.7	16.0	16	10
L20 2% + K-l-CB 1–50	15	24	0	0	0	3.7	14.8	15	7

[a] Explanation of abbreviations used in the table follows (for more details see p. 446):

Sulphur dusts

Oxidized sulphur = Sublimed sulphur plus potassium permanganate and a catalyst.
Precipitated sulphur = Derived from aerated lime sulphur.
Bentonite-sulphur = Sulphur fused with bentonite.

Explanation of abbreviations (*Continued*)

Sulphur spray
L-S = Commercial liquid lime sulphur.
Arsenicals
AL = Commercial powdered arsenate of lead.
CA = Commercial powdered arsenate of lime.
Copper fungicides
BM = Bordeaux mixture.
C-L-Ars. = Copper-lime-lead arsenate.
Emulsified oil sprays
L20, L202 } Emulsified oils.
L205 = L20 plus a form of sulphur.
Mercurial compounds
K-l-CB, K-l-GB } Proprietary organic ethyl mercury chloride compounds.
117, 117[c], 117[e] } Proprietary organic hexamethylene bichloride of mercury compounds.
118 = A pure monobasic mercurial compound.
Other materials
AS = Aluminum sulphate.
Kayso = Casein lime.
[b] Grh = Greenhouse. IC = Inoculation chamber.
[c] Neutral sodium soft soap (20th Century Soap).
[d] Abundance of the ascosporic inoculum was observed by microscopic examination of Petri plates exposed in representative situations in the chamber. The data refer to the approximate number of spores per low-power field of the microscope. The capital letters A and B differentiate the trees with respect to the amount of inoculum they received as indicated in the controls.
[e] Trees were given 12 hours in inoculation chamber immediately after treatment.
[f] The dusted trees were sprinkled with distilled water and then given moist treatment as in [e].
[g] The treated trees were sprinkled with distilled water 2 and 4 hours, respectively, after putting in inoculation chamber.
[h] Neutral potassium soft soap (XXX Cork Tile Soap).

periods of 48 and 81 hours at 6° C. in the moist chamber. Lime-sulphur preparations were the most effective in this inhibition. According to Greaney (26), who worked with certain cereal rusts, the length of time after inoculation during which control can be obtained with Kolodust is short and varies with the temperature, the period being from 10 hours at 10° to 12° C. to 5 hours at 22° to 24° C.

The present writer obtained excellent control of apple scab when treatments, particularly the sprays containing lime sulphur, followed soon after infection periods. Considerable differences were found in the effectiveness of the various fungicides when applied after inoculation. Sprays containing lime-sulphur were distinctly more effective than treatments of sulphur dusts (whether finely ground, sublimed, activated or containing arsenate of lead), wettable sulphur sprays, calcium monosulphide, Bordeaux mixture, emulsified oils with or without a sulphur fungicide, and certain proprietary mercurial compounds (Table 1, Ser. 6–15, and Table 2, Ser. 1–4, 7, 10, 11, 17, 18, 34, 36, 38). The greater toxicity exhibited by the sprays containing lime sulphur as compared with these other fungicides was dependent apparently neither on temperature within the range of 5° to 23° C. nor on the presence of high humidity after application. It appears that sulphur-arsenate dust, 90–10, offered little control when applied later than 12 hours following inoculations unless the application was followed by a moist treatment (Table 1, Ser. 6–15). Sprays containing lime sulphur, especially lime sulphur 1–40 plus arsenate of lead 1–50, gave good control when the application was made after infection periods ranging from 30 to 72 hours, and in some cases even longer (Table 1, Ser. 6–15; Table 2, Ser. 1–4, 7–11, 17–36; and Table 3, Ser. 3, 4). Lime sulphur-aluminum sulphate mixture was the most effective spray tested (Table 2, Ser. 8, 18, 19, 28). Lime sulphur 1–40 was not quite so effective as lime sulphur 1–40 plus arsenate of lead 1–50 (Table 2, Ser. 2, 7, 8, 11, 18, 19, 24, 31–35). This difference was decidedly more marked when a period in the moist chamber followed the application of the sprays. The results (Table 2, Ser. 24, 25) seem to be in general accord with field evidence that 1–40 is a satisfactory dilution for the lime-sulphur concentrate in apple-scab control. Under these conditions, there seemed to be no significant difference in fungicidal effectiveness attributable to their various physical states between the several wettable sulphur pastes or finely ground sulphur preparations and the less finely divided sulphur products (Table 1, Ser. 14, 15, and Table 2, Ser. 1, 2, 4, 5, 11, 13, 14, 16, 37). The amount of control obtained with precipitated lime sulphur appeared to be equal to that obtained with certain sulphur dusts and wettable sulphur pastes (Table 1, Ser. 14, 15, and Table 2, Ser. 5, 16). There is evidence that the wettable particulate sulphur pastes, which are by-products in the purification of

natural gas, contained a component other than sulphur, the toxic effect of which is increased in the presence of moisture. Calcium monosulphide, applied after infection periods of from 28 to 53 hours at 15° to 20° C., did not seem quite so toxic as lime sulphur 1–40 (Table 2, Ser. 9, 17, 18, 34). (Hurt and Schneiderhan (28).)

Bordeaux mixture, applied after infection periods of from 46 to 72 hours at 10° to 23° C., gave more consistent and better control than certain sulphur dusts and finely divided sulphur sprays (Table 2, Ser. 1–3). Emulsified oils, L202 and L20, appear to have fungicidal properties, although these may vary with the breaking point of the emulsion and with other factors (Table 2, Ser. 1, 3, 4, 38–40). L205, an emulsified oil containing a form of sulphur, did not appear to offer so good control as the sprays containing lime sulphur, although it seemed more toxic than oil emulsion alone, certain sulphur dusts and wettable sulphur sprays, and Bordeaux mixture (Table 2, Ser. 1–4, 38). Proprietary mercurial compounds, K–1–CB, K–1–GB, and 117E may be quite effective when applied 35 to 94 hours after inoculation (Table 2, Ser. 7, 17). K–1–CB is more effective than K–1–GB (Table 2, Ser. 7). In K–1–CB the toxic material is more soluble and volatile than in K–1–GB. K–1–CB, 117, 117C, 117E, and 118 (Table 2, Ser. 34, 38–40) decidedly increase the effectiveness of oil emulsion, L20, under these conditions and appear to warrant further investigations as fungicides for oil emulsions.

Kolodust, lime sulphur 1–40 plus arsenate of lead 1–50, and lime sulphur 1–40, applied 12, 46, and 32 hours, respectively, after inoculation began, show that the quantity of ascosporic inoculum was an important factor in the effectiveness of the sulphur fungicides (Table 2, Ser. 15, 22, 23). Further data of the same general trend appear in the other series in table 2.

The question arises as to the feasibility of applying fungicides after infection periods in the field. Since no orchard spraying experiments were included in the present investigation this point can not be discussed in detail. However, it may be recorded that unpublished results made available to the writer by Keitt and Wilson are in conformity with those obtained in the greenhouse and show striking effectiveness of lime sulphur plus lead arsenate in controlling apple scab when applied soon after an infection period. From the evidence at hand it would appear that prompt spraying with lime sulphur and lead arsenate after critical infection periods should be of much value in emergency situations in which the trees have not been adequately protected during such periods.

RELATION OF TEMPERATURE TO THE EFFECTIVENESS OF SULPHUR FUNGICIDES

Considerable theoretical and practical interest attaches to the relation of temperature to the fungicidal action of sulphur preparations. Marès

(33) wrote that sulphur will destroy the powdery mildew of the grape wherever the sulphur touches it, provided the temperature is not below 20° C. Doran (10) concluded that the toxicity of the sulphur to fungi increases with rise of temperature and length of time of exposure. He reported that sulphur dust was toxic to the conidia of *Venturia inaequalis* when the temperature remained at 26° C. for five hours. Butler (7) considered that temperature was an important factor in the effectiveness of sulphur in the control of snapdragon rust. He reported that sulphur was quite ineffective at temperatures below 15° C. Tisdale (52) stated that the toxicity of colloidal sulphur (prepared from sulphur dioxide and hydrogen sulphide) to *Botrytis cinerea* Pers. was greater at the higher temperatures tested. According to Butler and Doran (8), the toxicity of lime-sulphur solution depends on the temperature preceding spore germination. Goodwin and Martin (24) found that the amount of a volatile sulphur derivative formed from sulphur increased with a rise in temperature when tested by a copper-foil method. However, these tests were feasible only at comparatively high temperatures. In a later paper Goodwin and Martin (25) offered evidence to indicate that the production of gaseous sulphur at ordinary temperatures was insufficient to be effective against the conidia of *Sphaerotheca humuli* (DC.) Burr and *Erysiphe graminis* DC., although sulphur is known to be toxic to these two fungi. They found, however, that the gall mite, *Eriophyses ribis,* was affected by the traces of sulphur volatilized at ordinary temperatures. It is evident from the data of Keitt and Jones (29) that various sulphur treatments including sulphur dust alone were effective against *V. inaequalis* at 6° C. Greaney (26) found that a temperature as low as 12° C. had very little influence upon the effectiveness of sulphur dust when applied before infection in the control of some cereal rusts but reported that the length of time during which control could be obtained when applications were made after inoculation varied with the temperature. At temperatures of 10° to 12° C. sulphur prevented any serious infection when applied 10 hours after inoculation, while at the higher temperatures, 22° to 24° C., it was equally effective when applied 5 hours after inoculation. The increased period of effectiveness at the lower temperatures was accounted for by the slower development of the rust fungus in that environment rather than by an increase in activity of the sulphur. As the result of an investigation of the control of brown rot of peaches in storage with Koppers sulphur dust, Smith (48) reported that definite fungicidal action was exerted by sulphur at 40° F. However, he obtained no control of brown rot at temperatures of 65° F. and above. The relation of temperature to the growth of the pathogene should be considered in interpreting these results.

TABLE 3.—*Relations of temperature and humidity to the effectiveness of Kolodust and sprays containing lime sulphur when applied after inoculation of Wealthy apple leaves with the ascospores of Venturia inaequalis*

Series, date, and fungicide[a]	Period in IC[a] at 15° C.	Period in Grh[a] before fung. app.	Subsequent treatment of plant[a]	Final results averaged per twig			
				Leaves infected	Max. lesions on one leaf		Total lesions
	Hrs.	Hrs.		No.	No.	Rel. No.	Rel. No.
Series 1,[b] 3–20–29							
Untreated	24	0	24 hrs. 18°–55% Grh[a]	5.3	83.0	100	100
Kolodust	24	0	"	4.7	77.7	94	62
Untreated	24	0	24 hrs. 28°–50% Grh	5.0	65.7	100	100
Kolodust	24	0	"	5.0	37.3	57	57
"	24	0	" [e]	3.3	23.3	35	32
Untreated	24	0	24 hrs. 28°–80% Grh	4.0	76.5	100	100
Kolodust	24	0	"	2.7	17.0	22	22
"	24	0	" [e]	3.0	11.0	14	15
Untreated	24	0	Intermittent 28°–80% Grh[d]	5.0	28.0	100	100
Kolodust	24	0	"	3.3	12.3	44	38
Series 2,[ce] 3–22–29							
Untreated	4	0	18°–55% Grh[a]	5.2	85.0	100	100
Kolodust	4	0	6 hrs. 18°–55% Grh	3.8	37.3	44	39
"	4	0	9 hrs. 18°–55% Grh	2.2	10.2	12	12
"	4	0	18 hrs. 18°–55% Grh	2.3	2.5	3	2
Untreated	4	0	9 hrs. 28°–80% Grh	5.0	36.2	100	100
Kolodust	4	0	3 hrs. 28°–80% Grh	3.2	9.4	26	28
"	4	0	6 hrs. 28°–80% Grh	3.0	6.3	17	9
"	4	0	9 hrs. 28°–80% Grh	1.0	1.1	3	2
Series 3, 4–13–29							
Untreated	48	24	18°–55% Grh[a]	7.0	62.0	100	100
L-S 1–40	48	24	"	5.3	92.3	149	87
L-S 1–40 + AL 1–50	48	24	"	5.6	54.0	87	43
Untreated	48	24	24 hrs. 26°–47% Grh	7.0	62.5	100	100
L-S 1–40	48	24	"	3.8	15.0	24	14
L-S 1–40 + AL 1–50	48	24	"	4.0	19.5	31	15
"	48	24	48 hrs. 26°–47% Grh	2.7	15.0	24	9

TABLE 3—(*Continued*)

Series, date, and fungicide[a]	Period in IC[a] at 15° C.	Period in Grh[a] before fung. app.	Subsequent treatment of plant[a]	Final results averaged per twig			
				Leaves infected	Max. lesions on one leaf		Total lesions
	Hrs.	Hrs.		No.	No.	Rel. No.	Rel. No.
Series 3, 4–13–29—Continued							
Untreated	48	24	48 hrs. 26°–83% Grh	7.0	59.0	100	100
L-S 1–40 + AL 1–50	48	24	"	2.5	7.0	12	7
Series 4, 4–16–26							
Untreated	45	27	18 hrs. IC, 18°–55% Grh[a]	7.2	100.0	100	100
L-S 1–40 + AL 1–50	45	27	"	3.3	34.8	35	12
Untreated	45	27	26 hrs. 30°–80% Grh	4.0	27.5	100	100
L-S 1–40 + AL 1–50	45	27	6.5 hrs. 30°–80% Grh	2.5	38.8	141	58
"	45	27	18 hrs. 30°–80% Grh	1.3	2.5	9	7
"	45	27	26 hrs. 30°–80% Grh	0.0	0.0	0	0

[a] Temperature in °C. Relative humidities in per cent. Grh = Greenhouse. IC = Inoculation chamber. L-S = Commercial liquid lime sulphur. AL = Commercial powdered arsenate of lead. The plants were incubated, except as otherwise stated, in a greenhouse which was approximately 18° to 23° C. and 50 to 60% relative humidity, without effort to control humidity.

[b] Series was given 24 hours in the inoculation chamber following the treatment above.

[c] Thoroughly washed with a spray of lake water following the temperature treatment.

[d] Six periods of 12 hours each were alternated between 28°–80% and 20°–50% greenhouses, respectively.

[e] Series was in the inoculation chamber for 48 hours at 15° C. 18 hours after inoculation.

Good control was obtained with sulphur dusts and sprays applied before inoculation when temperatures of from 5° to 24° C. were maintained during the infection period (Table 1, Ser. 1–5, 8, 9, 11–18; Table 6, Ser. 1–7; and Table 7, Ser. 1–3). Kolotex gave complete control at 2.5° C. (Table 1, Ser. 16); but, as is shown later, this may have been due in part to the arsenate of lead. Fungicidal applications made after infection show that temperatures within the range of from 6° to 23° C. during an infection period did not materially affect the control of *Venturia inaequalis* (Tables 1 and 2). Temperatures of 26° C. and above, during the period following the discharge of ascospores, may be an important factor in the amount of control (Table 3, Ser. 1–4). It appears that the amount of infection obtained in the controls was determined by the temperature and length of exposure to the temperature, if this was near the upper limit of toleration for the fungus. It is apparent that high temperatures were more effective in preventing infection than in checking the organism after it had become well established in the host. Temperatures above 26° C. markedly inhibited infection by *V. inaequalis*. Brief exposures to a temperature of 28° C. distinctly increased the effectiveness of Kolodust applied 24 hours after inoculation (Table 3, Ser. 1). Kolodust, applied after an infection period of 4 hours at 15° C., effected as much control in 9 hours at 28° C. as in 18 hours at 18° C. (Table 3, Ser. 2). It will be noted that the beneficial result of a rise in temperature on the effectiveness of sulphur dust was more marked when the treatment was made soon after inoculation. The control obtained with lime sulphur 1–40 and lime sulphur 1–40 plus arsenate of lead 1–50, applied 72 hours after inoculation, was decidedly greater at 26° to 30° C. than at 18° C. (Table 3, Ser. 3, 4). The effectiveness of the spray increased markedly according to the length of the exposure to the higher temperatures. It is evident from these experiments that consideration must be given to the effect of temperature, not only on the action of the sulphur fungicide but also on the growth of the fungus and of the host and on the host-parasite relation.

The minimal number of hours of continuous wetting necessary for infection by ascospores of *Venturia inaequalis* was reported by Keitt (30) as follows: 6°, 15; 9°, 11; 15°, 7; 20°, 4; and 24° C., 6.

THE RELATION OF MOISTURE TO THE EFFECTIVENESS OF CERTAIN FUNGICIDES

Doran (9) reported that sulphur was nontoxic to the urediniospores of *Puccinia antirrhini* Dietl. & Holw. when applied in water but was toxic when applied dry. Barker, Gimingham, and Wiltshire (2) observed that spores of *Sclerotinia fructigena* Pers., on the outside of the film of a hanging drop, did not germinate when dry precipitated sulphur was added to

the outside of the drop, but spores that were wet and remained suspended in the center of the drop germinated well. Yossifovitch (64) considered that the action of sulphur in the control of Oidium of the vine was diminished by humidity. Young (61), using the spores of *S. cinerea* with a hanging-drop technique, concluded that a certain water requirement was necessary for sulphur to be effective. Greaney (26), in a study of the control of some cereal rusts, found that the effectiveness of sulphur was greatly reduced when free moisture was abundant either before or after inoculation. The relation of moisture to the passage of a volatile substance across space was investigated by Goodwin and Martin (24), who made a quantitative estimate of the stain produced by the reaction of a volatile material from sulphur with copper. They found that the influence of humidity on the production of the stain was negligible, although there was evidence that its formation was favored in the absence of moisture.

A period of 12 hours in the moist chamber, ending 6 hours before inoculation, did not have a noticeable inhibitory effect on the fungicidal action of sulphur dusts and sprays applied 24 hours before inoculation (Table 1, Ser. 1–3, 8, 9, 11–14). Aerated lime sulphur 1–40, Koloform, Kolodust, and Super-sulfodust, applied 30 to 48 hours after inoculation, did not prevent further infection when the treated plants were placed in the moist chamber for 15 to 20 hours at 10° to 14° C. (Table 1, Ser. 14, and Table 2, Ser. 11, 12). However, applications of Kolodust, made following periods in the moist chamber of from 12 to 45 hours at 15° to 20° C., indicate that a moist treatment increased the amount of control (Table 2, Ser. 13–16), although there might be at the same time actually an increase in the amount of infection in treated trees. It should be noted that the controls (Table 1, Ser. 14, Table 2, Ser. 11, 12) were in the moist chamber only during the infection period. Had they received the second moist treatment along with the treated trees, it is probable that more disease would have developed. This fact may account for what appears to have been a decrease in toxicity incident to subjecting the treated plants to the moist period. It is evident that the sulphur was not sufficiently toxic under these conditions to prevent further development of the fungus. The degree to which moisture effected an increase in the toxicity of the sulphur was apparently greater when the application was made a comparatively short time after inoculation. A surplus of water applied to the sulphur after the application did not tend materially to affect its toxicity (Table 2, Ser. 15). Moist treatments, ranging from 15 hours at 5° C. to 20 hours at 10° C., following fungicidal applications, markedly increased the effectiveness of sulphur-arsenate dust 90–10 (Table 1, Ser. 12–14); Kolotex (Table 2, Ser. 10, 11); arsenate of lead (Table 2, Ser. 11, 16); Bentonite-sulphur 5–50 plus arsenate of lead

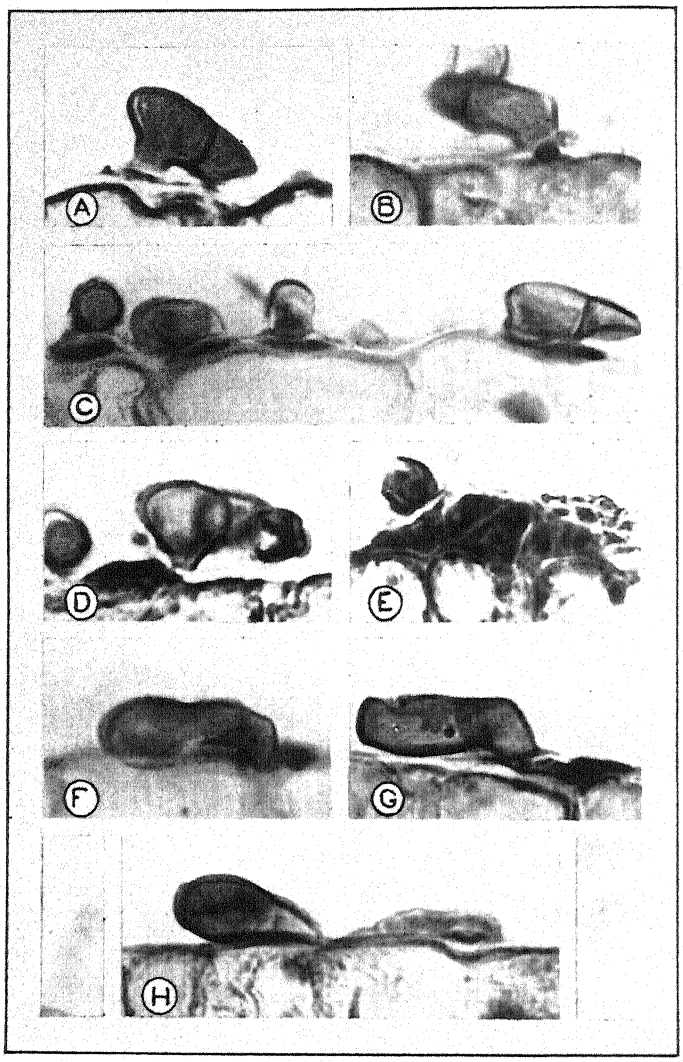


FIG. 3. Penetration of *Venturia inaequalis* into the ventral surface of Wealthy apple leaves in relation to the action of certain fungicides. All figures × 1016. A. Germinated ascospore after 7 hours in moist chamber at 20° C. No penetration. B. Infection established after 10-hours incubation at 17° C. C. Penetration after 24-hours incubation at 17° C. D. Typical infection of leaf treated with Kolodust after 10-hours incubation at 17° C., followed by 14-hours moist treatment at 17° C. before fixation. E. Subcuticular development of fungus after 50-hours incubation at 20° C. Plants then sprayed with lime sulphur 1–40 plus arsenate of lead 1–50 and given 18-hours moist treatment at 20° C. F, G. Penetration from conidia after 24 and 30 hours, respectively, incubated at 15° C. H. An appressorium formed by a conidium after 24-hours incubation at 15° C.

1–50 (Table 2, Ser. 17); calcium monosuphide 10–50 plus arsenate of lead 1–50 (Table 2, Ser. 17, 34); calcium monosulphide 10–50 (Table 2, Ser. 18,[5] 34); Ferrox sulphur paste 5–50, Thylox sulphur paste (unwashed) 5–50, and Gray sulphur paste 5–50 (Table 2, Ser. 13, 14); and stone lime 3–50 (Table 2, Ser. 16). The fungicidal actions of lime sulphur 1–40 plus arsenate of lead 1–50 (Table 1, Ser. 12–14, and Table 2, Ser. 10, 11, 18–22, 27, 35); lime sulphur 1–40 plus calcium arsenate 1–50 (Table 2, Ser. 18–21); and lime sulphur 1–40 (Table 1, Ser. 14, and Table 2, Ser. 11, 18,[5] 19,[5] 35) were also decidedly increased by similar treatments. The increased inhibiting effect of lime sulphur 1–40 plus arsenate of lead 1–50, due to a moist treatment following its application, was dependent upon the duration of the treatment (Table 2, Ser. 22, 27). An increase in humidity below the saturation point at 26° C. or above appears to aid the sulphur dusts and sprays containing lime sulphur in checking the fungus (Table 3). Other indications of the relation of moisture to the efficiency of the various sprays may be found by further study of table 2.

HISTOLOGICAL STUDIES ON PENETRATION OF THE FUNGUS IN RELATION TO THE ACTION OF CERTAIN FUNGICIDES

Studies were made of infection of leaves of potted Wealthy apple plants at various intervals after inoculation with conidia and ascospores of *Venturia inaequalis*. The conidia were washed from abundantly sporulating lesions on freshly collected apple leaves and sprayed upon the experimental apple leaves by means of an atomizer. The ascospores were ejected from perithecia in overwintered apple leaves directly upon susceptible leaves that previously had been wetted. Inoculations were made on the upper side only. Plants with leaves of approximately the same age and texture were used. Infection was accomplished in the moist chamber. Portions of inoculated leaves at the desired stages of infection or after certain fungicidal treatments were placed in formal-acetic alcohol, and parowax serial sections were prepared from them. Safranin and fast green proved to be the most successful combination of stains tried. The safranin stained the fungal hyphae within the host red, and the fast green stained the cuticle and epidermal layer of cells of the leaf green, thus giving a striking contrast.

Studies of the fixations of the conidial material supported, in general, the results of Keitt and Jones (29) and those of certain workers quoted by them. The well-differentiated appressorium (Fig. 3, H), considered a typical development before infection, was somewhat uncommon under these conditions as compared with the relatively short, thick germ tubes (Fig. 3,

[5] These data are inconsistent with the general trend. The period which elapsed before the moist treatment was given appears to have been a factor.

F, G) that appeared to function as appressoria without the formation of clearly differentiated holdfasts. Sections F, G, and H (Fig. 3), referred to above, were prepared from fixations made after infection periods of 24, 30, and 24 hours, respectively, at 15° C. When ascospores were used as inoculum, infection was not preceded in the majority of cases by the development of a well-differentiated appressorium. This was especially true if the spores became situated on susceptible leaf surface. Modifications of the germ tube have been noticed in cases where for some reason the germ tube did not originate or develop near the leaf surface but came in contact with it some distance from the spore. The ascospore itself (Fig. 3, A, B, C, D) may function as an appressorium from which an infection hypha may penetrate directly into the cuticle, as reported by Keitt and Jones (29). However, it is not uncommon to find comparatively short, thick germ tubes (Fig. 3, A, B, D) which probably function as holdfasts. As pointed out by Keitt and Jones (29), this type of infection is important in relation to the effectiveness of fungicides, as it shortens the time necessary for infection and reduces the area exposed to the action of a toxic agent before infection occurs. Sections (Fig. 3, A, B, C), made after infection periods of 7, 10, and 24 hours, respectively, at 17° C., show the different stages of infection under these conditions. Penetration was not observed for an infection period of less than 10 hours. It is evident from a comparison of B and D (Fig. 3) that Kolodust, applied after an infection period of 10 hours, was ineffective when the application was followed by a period in the moist chamber of 14 hours at 17° C. The stage of infection shown in figure 3, D, is as far advanced as that of figure 3, C, in which no sulphur dust was applied. Figure 3, E, shows a stage of infection following a period of 50 hours at 20° C. in the moist chamber after inoculation began. Lime sulphur 1–40 plus arsenate of lead 1–50, applied at this time and followed by a moist treatment of 18 hours, gave good control.

DISCUSSION

It would seem from the data that have been presented that sulphur dusts gave an initial control as great as that of the sprays when applied before inoculation and left in place. It is apparent, as found by Keitt and Jones (29), that the various sulphur treatments with or without arsenate of lead are effective at a temperature as low as 6° C. This accords with their field data and with the results of others who have obtained satisfactory control of apple scab at comparatively low temperatures in the spring. It is against the view of those investigators who have maintained that finely divided sulphur is not effective at these low temperatures. It is evident that the subjection of a sulphured plant to a period in the

moist chamber before inoculation has no inhibitory effect on the fungicidal action of sulphur provided there is no washing action, and that, if moisture or water is an essential for sulphur to be effective, there was a sufficient quantity available during the infection periods.

The data show that it is possible to control *Venturia inaequalis* soon after infection by applying certain fungicides, notably sprays containing lime sulphur. Other sprays and dusts tested are markedly less effective. Sprays containing lime sulphur have repeatedly given control when applied after intervals ranging from 30 to 72 hours or even longer after inoculation, while sulphur-arsenate dust has not been so effective after 12 hours. Histological evidence would indicate that sulphur dust had no appreciable effect on *V. inaequalis* after infection was established, while lime sulphur plus arsenate of lead was effective after considerable subcuticular development of the fungus. Temperature is apparently an important factor in the inhibition of the fungus (through its relationship to host and parasite) and in the amount of control effected by the sulphur fungicide. Moisture, in part, determines the maximum control obtainable with the use of many fungicides, particularly those containing an arsenical compound. From the evidence at hand it would appear that prompt spraying with lime sulphur and lead arsenate after critical infection periods should be of much value in emergency situations in which the trees are not adequately protected during such periods.

OTHER STUDIES CONCERNING THE EFFECTIVENESS OF CERTAIN FUNGICIDES

During the course of these studies it has not been feasible to make more than an exploratory investigation of some factors which bear upon the control obtained with certain fungicides A brief record of the results of this phase of the investigation follows.

Time required for sprays containing lime sulphur and Kolodust to be effective when applied after inoculation. The nature of fungicidal effectiveness and the time required for toxicity to be exhibited are of material significance in the evaluation of a spray or dust. The toxicity of a fungicide is generally expressed as a killing or an inhibitory action. A consideration of the latter phase of toxicity involves the question as to whether the fungus is continuously held in check by the fungicide or only inhibited long enough for the host to outgrow it or become resistant to its invasion. The time required for sulphur to be effective when applied after an infection period of 4 hours was determined, at least in part, by the temperature that followed the application (Table 3, Ser. 2). While Kolodust may require 18 hours to be effective at a temperature of 18° C., it may exhibit the same degree of toxicity in 9 hours at 28° C. The amount of infection on the control for the 28° C. treatment was markedly reduced as compared to that of the control held at 18° C.

TABLE 4.—*The rate of fungicidal action of Kolodust and lime sulphur 1–40 applied after inoculation of Wealthy apple leaves with the ascospores of Venturia inaequalis*

Series, date and fungicide[a]	Period in IC[a] at 16° C.	Period in Grh[a]	Period for fung. action[b]	Incubated in IC at 16° C.	Final results averaged per twig			
					Leaves infected	Max. lesions on one leaf		Total lesions
	Hrs.	Hrs.	Min.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 1, 6–4–28								
Untreated	2	1	0	44	0.7	1.0	.....	.....
Kolodust	2	1	10	44	0.3	2.0	.....	.....
"	2	1	60	44	0.3	0.3	.....	.....
Untreated	9	0	0	44	2.5	2.5	.....	.....
Kolodust	9	0	10	44	1.8	3.5	.....	.....
"	9	0	60	44	1.0	1.5	.....	.....
Series 2, 6–12–28								
Untreated	0	0	0	30	6.2	63.0	100	100
"	2	1	0	30	2.0	8.0	13	5
"	3	0	0	30	3.5	34.5	55	35
L-S 1–40	2	0	10	30	0.0	0.0	0	0
"	2	0	60	30	0.0	0.0	0	0
Untreated	9	0	0	30	6.0	73.0	100	100
L S 1–40	9	0	10	30	0.0	0.0	0	0
"	9	0	60	30	0.7	4.0	6	3
Series 3, 3–7–29								
Untreated	0	0	0	48	6.3	100.0	100	100
"	2	0	0	48	3.2	20.5	21	12
"	2	1	0	48	4.3	49.5	50	18
"	4	0	0	48	4.0	55.0	55	32
"	4	1	0	48	6.5	100.0	100	109
"	6	0	0	48	5.3	100.0	100	95

[a] L-S = Commercial liquid lime sulphur. Grh = Greenhouse. IC = inoculation chamber.
[b] After stated intervals the leaves were thoroughly washed with a spray of lake water.

Lime sulphur 1–40 gave control if it remained on the foliage for a period of 10 minutes at 16° C., when applied following periods of 3 and 9 hours, respectively, after inoculation (Table 4, Ser. 2). Although the trees were given a thorough washing with a spray of water, it is possible that the foliage or the spores still retained some of the lime sulphur. The trees were kept in the moist chamber from the time of inoculation to that of washing, thereby preventing the drying of the lime sulphur. Considerable infection occurred when lime sulphur 1–40, applied at the end of an infection period of 48 hours at 16° C., was washed off after 4½ hours in the moist chamber (Table 2, Ser. 25, 26). It required about 8 hours in the moist chamber for maximum effectiveness to be exhibited under these conditions. Lime sulphur 1–40 plus arsenate of lead 1–50, applied after an infection period of 75 hours, did not fully inhibit the fungus after a moist treatment of 18 hours (Table 2, Ser. 27). Lime sulphur 1–40 plus arsenate of lead 1–50, applied 72 hours after inoculation, gave very poor control when the trees were incubated at 18° C. immediately following treatment (Table 3, Ser. 4). Excellent control was obtained, however, when the treated trees were incubated at 30° C. for 18 hours before removal to the lower temperature. The effect of a somewhat similar high-temperature treatment on a control was no less striking. It is evident that the time required for the maximum amount of control to be exhibited by sulphur fungicides under these conditions is markedly influenced by temperature.

Little infection occurred even in the case of the control when inoculated foliage was washed at the time of inoculation or shortly after (Table 4, Ser. 1). Inoculated trees exposed 2 hours in the moist chamber had nearly all of the spores removed by washing, although the leaves were allowed to dry 1 hour before they were washed (Table 4, Ser. 2). It is evident that after 3 hours or longer in the moist chamber, washing removed decidedly fewer spores. In the case of a heavier inoculation more spores adhered, though the general trend was the same (Table 4, Ser. 3). The spores were more adhesive when the leaves were dried 1 hour before washing (Table 4, Ser. 3). It would seem to follow that at about this time appressoria or attachments had developed. From these and other data it appears that the length of time after inoculation during which the spores may be washed off is variable. This probably is because of variations in the position of the spores in relation to the leaf surface, the condition of the spores, the type of foliage, and other environmental conditions.

Relation of the rate of drying of lime sulphur plus arsenate of lead spray to its effectiveness. In tests with the conidia of *Venturia inaequalis* in the laboratory, Doran (10) found that when lime-sulphur sprays were dried rapidly, undecomposed polysulphides were present and spore germination was prevented. However, if desiccation was slow, the polysulphides

were completely decomposed and the preparations were only slightly toxic or nontoxic under the conditions of his experiments. Butler and Doran (8) reported that sulphides were absent from lime sulphur 1–40, which had been held in a moist chamber at room temperature 3½ hours. High humidity, which checked evaporation, hastened decomposition.

The data of table 2 (Ser. 28, 29) are in agreement with Doran's report in that there was reduced effectiveness with slow drying. The treatments were made following periods of 66 to 72 hours, respectively, in the moist chamber after inoculation began. Lime sulphur 1–40 plus arsenate of lead 1–50, dried with a fan after application, showed increased effectiveness as compared with similar treatments allowed to dry in the greenhouse or kept in the moist chamber a short time after application. Incubation of the treated trees in the moist chamber at 10° C. for 2 to 4 hours apparently inhibited the toxic action. It appears, therefore, that the amount of moisture or humidity present during the early period after application of the spray is a factor in its effectiveness when applied after inoculation.

Relation of alkalinity to the initial effectiveness of the sprays containing lime sulphur. Foreman (12) concluded that free "alkali soda" is the most potent fungicidal agent of liver of sulphur. Eyre and Salmon (11) were unable to substantiate this contention. They found a solution containing 0.5 per cent caustic soda plus 1 per cent soft soap to be nonfungicidal against *Sphaerotheca humuli.* Barker, Gimingham, and Wiltshire (2) reported that it is not the alkalinity of the sulphide or the polysulphides of sodium that gives them their toxicity, although they stated that it may aid the toxic action. Young (61) suggested that the initial toxicity of lime-sulphur mixtures may be due partly to alkalinity or free hydroxyl ions. He reported the alkalinity of 1 part of lime sulphur diluted with 6 parts of water to be above pH 10. The data of Young (61) show that there was almost no germination for *S. cinerea* at pH 7.4 in a buffered mixture. He reported that the lime sulphur 1–6 changes from pH 10 to pH 6.4 in 2 hours if exposed to air, but may not change below pH 10 for 6 hours or longer if kept moist.

Sodium hydroxide, N/10 solution (Table 2, Ser. 12, 25, 28) and sodium hydroxide N/14 solution (Table 2, Ser. 16, 25) were strikingly effective and compared favorably with sprays containing lime sulphur when applied 16 to 66 hours after inoculation began. A moist treatment of 18 hours did not significantly affect the amount of control exhibited by sodium hydroxide N/10 solution (Table 2, Ser. 12). It is apparent that, at least as far as the effectiveness of lime sulphur applied after inoculation is concerned, alkalinity is probably an important factor in its toxicity.

Relation of the addition of lime to the effectiveness of certain sulphur fungicides. Swingle (49) reported that in storage varying quantities of

calcium arsenate are formed in different sulphur-lead-arsenate-lime dusts and that all the hydrated lime not reacting with the sulphur or lead arsenate is converted into calcium carbonate within one year or less. He concluded from chemical analysis that little or no change in fungicidal value should be expected. Reports vary as to whether or not the addition of lime to lime sulphur plus arsenate of lead increases or decreases its fungicidal value. As a result of a limited number of germination tests made on glass slides with the spores of *Sclerotinia fructigena* and of certain field tests, Wallace, Blodgett, and Hesler (56) were convinced that the addition of lime does not affect the efficiency of lime sulphur and lime sulphur plus arsenate of lead solutions to any marked degree. Robinson (46) reported that the addition of lime to a lime sulphur-arsenate of lead solution prevented arsenic from going into solution as a soluble salt and decreased the loss in polysulphides, thereby preserving the fungicidal properties of the lime sulphur. Thatcher and Streeter (50 state that hydrated lime prevented the chemical reactions which take place when arsenate of lead is added to lime sulphur. In their estimation these reactions reduce the fungicidal efficiency of the mixture. There appears to be no doubt that the addition of lime to the lime-sulphur-arsenate of lead preparation reduces the amount of water-soluble arsenic by the production of insoluble basic calcium arsenate, as shown by Mogendorff (37), Ginsburg (15), Van Der Meulen and Van Leeuwen (53), and Goodwin and Martin (23).

Hydrated or stone lime 3–50, applied as spray, 16 to 90 hours after inoculation and followed by moist treatments ranging from 10 to 13 hours, may exhibit fungicidal properties of their own under certain conditions (Table 2, Ser. 6, 16, 31). Indications were that the effectiveness of at least the stone lime was dependent in part upon the moist treatment following the application (Table 2, Ser. 16). Lime, whether hydrated or stone, exhibited for the most part comparatively little toxicity when applied as a spray before inoculation (Table 6, Ser. 8–10, and Table 7, Ser. 3, 4). Kolotex 8–50, applied with lime 3–50 as a spray after an infection period of 72 hours, was distinctly more effective than Kolotex, applied as a dust (Table 2, Ser. 36). The application of lime sulphur 1–40 plus arsenate of lead 1–50 and lime 3–50, following infection periods of from 66 to 90 hours, would indicate that on the average the lime did not materially decrease the effectiveness of lime sulphur plus arsenate of lead (Table 2, Ser. 28, 30, 31).

Relation of the addition of arsenate of lead to the effectiveness of lime sulphur and certain sulphur dusts. Morse (39, 40, 41) found that under favorable circumstances arsenate of lead has fungicidal value for the control of *Venturia inaequalis* under field conditions. However, as shown by Pickett et al. (43), Wallace (55), Morse (41), and Morse and Folsom (42),

arsenate of lead, although exhibiting fungicidal properties, is not satisfactory as a fungicide. It would appear that the effectiveness of arsenate of lead is dependent on the severity of the epiphytotic. Tests made in the laboratory with *V. inaequalis* by Shapovalov (Morse, 39), Wallace, Blodgett, and Hesler (56), and Butler and Doran (8) confirmed the conclusions to be drawn from field experiments that arsenate of lead is not completely effective at the strength used in orchard spraying.

Wallace, Blodgett, and Hesler (56) proved conclusively that the addition of lead arsenate does not decrease the fungicidal value of lime-sulphur solution but greatly increases it. Their tests were made in the laboratory with the conidia of *Venturia inaequalis, Sclerotinia fructigena,* and *Sphaeropsis malorum* Adehr., while the field tests were conducted to control *V. inaequalis* and *V. pyrina* Aderh. Bradley and Tartar (5) and several other workers have determined that soluble arsenic is formed in mixtures of lime sulphur and acid lead arsenate. More recently it has been reported by Goodwin, Martin, and Salmon (18) that solutions of calcium polysulphide and lead arsenate containing 0.204 per cent arsenic pentoxide are fungicidal. Butler and Doran (8) conducted experiments on glass with the spores of *V. inaequalis,* which show that the toxic properties of lime-sulphur solution are increased by the addition of arsenate of lead and that this in all probability is because of soluble arsenic. Marked increase in toxicity was obtained by the addition of arsenious oxide to the 1 in 40 lime-sulphur solution. They found that lead arsenate increases the toxicity of lime-sulphur solution decidedly more than calcium arsenate. Goodwin, Martin, and Salmon (18, 19) suggested that the increased fungicidal properties of the mixed lime-sulphur-lead arsenate spray are due to the presence of calcium thioarsenates together with calcium arsenates. Smith (47) presented data to show that the excretions from leaves containing large quantities of salts are significant factors in increasing the water-soluble arsenic content of sprays containing arsenical compounds. He found that all plants do not excrete the alkaline salts responsible for this change. This effect of alkaline salts is supported by the work of Mogendorff (37) and others cited by him. Ginsburg (13) reported that apparently high humidity, intense sunlight, and a high temperature acting separately or together do not play any significant rôle in the liberation of water-soluble arsenic. Mogendorff (37) found that water-soluble arsenic was formed when lead arsenate and sulphur flour were stored dry for 1 year. However, Ginsburg (15) reported that sulphur alone does not materially influence the decomposition of acid-lead arsenate.

In the present work arsenate of lead 1–50 or 2–50 was relatively ineffective when applied before inoculation (Table 6, Ser. 8, 10). Arsenate of lead used separately as a spray after infection periods of 16 to 72 hours was

not strikingly effective (Table 2, Ser. 2, 3, 11, 16). The fungicidal effect of arsenate of lead may be increased by a moist treatment after inoculation. The addition of arsenate of lead to Kolodust (Kolotex) may show an actual decrease in control as compared to Kolodust alone when applied after an infection period of 46 hours without a subsequent moist treatment (Table 2, Ser. 2). However, a marked increase in effectiveness for sulphur dusts containing arsenate of lead occurred when moist treatments of 15 to 20 hours followed the applications to foliage after infection periods of from 15 to 48 hours (Table 1, Ser. 12–14, and Table 2, Ser. 10, 11).

The effectiveness of calcium monosulphide, when applied after infection periods of from 28 to 35 hours, also was increased by the addition of arsenate of lead (Table 2, Ser. 9, 17, 34). The data of table 2 (Ser. 2, 7, 8, 11, 18, 19, 24, 32–35), where all the series except 2 and 24 were given periods in the moist chamber of rfom 10 to 20 hours and where lime sulphur 1–40, with and without arsenate of lead 1–50, was applied after infection periods of from 28 to 90 hours, show a similar trend of increased fungicidal effectiveness resulting from the addition of arsenate of lead. While the addition of calcium arsenate 1–50, (Table 2, Ser. 18–21) to lime sulphur 1–40, 60 to 82 hours after inoculation, did not increase its effectiveness as did the arsenate of lead, it did markedly increase the toxicity when the applications were followed by moist treatments of 8 to 12 hours. The results obtained from the use of basic arsenate of lead were not widely different from those secured with the addition of acid arsenate of lead (Table 2, Ser. 32). However, this test was too limited to be conclusive.

From a limited series of experiments (Table 2, Ser. 32–35), conducted to gain evidence on the part played by soluble arsenic in the fungicidal action of lime-sulphur spray containing arsenate of lead, it appears that if As_2O_5 is added in sufficient quantity it can increase the efficiency of the lime-sulphur spray as much as the addition of arsenate of lead. The addition of 0.01 per cent As_2O_5 to lime sulphur 1–40 did not increase its toxicity (Table 2, Ser. 32). Very little increased fungicidal action resulted when 0.02 per cent As_2O_5 was added to lime sulphur 1–40 and applied 48 hours after inoculation (Table 2, Ser. 33). However, 0.02 per cent of As_2O_5 was apparently as effective as arsenate of lead 1–50 in increasing the toxicity of lime sulphur 1–40, applied after an infection period of 28 hours (Table 2, Ser. 34). The addition of 0.03 per cent of As_2O_5 to lime sulphur 1–40 was likewise as effective as arsenate of lead 1–50 in increasing the fungicidal action of lime sulphur when applied 35 hours after inoculation (Table 2, Ser. 35). The As_2O_5, 0.03 per cent, was as effective alone as the lime sulphur 1–40 (Table 2, Ser. 35). There was no host injury when 0.04 per cent As_2O_5 was added to the lime sulphur 1–40. Unless otherwise stated, sterile distilled water was used in the preparation of the spray

materials in these experiments. When alkaline water is used, less soluble arsenic in the spray would be expected. Table 2 (Ser. 20, 21) and other data tend to show that the alkalinity of the water used in the preparation of lime sulphur 1–40 plus arsenate of lead 1–50 or calcium arsenate 1–50 was a factor in reducing fungicidal effectiveness when applied after infection periods of from 60 to 75 hours. The spray prepared from lake water, about pH 8, tended to be less toxic than that made with distilled water of about pH 5.7.

Relation of the addition of some spreaders and adhesive materials to the effectiveness of certain fungicides—Casein lime.—Thatcher and Streeter (50) found that the addition of casein-containing preparations effectively prevents the chemical change that occurs when acid lead arsenate is mixed with lime-sulphur solution. They reported that the data available from orchard experiments indicate that there is little likelihood of a reduction in the insecticidal and fungicidal effectiveness of the combined lead arsenate and lime-sulphur sprays as a result of its use. Mogendorff (37) and Ginsburg (15) pointed out that the addition of calcium caseinate to acid lead arsenate spray increases the water-soluble arsenic content. The former found, however, that the decomposition of the lead arsenate was greatly diminished and that in a complete dry mix spray the presence of the casein-lime spreader together with the lime and sulphur greatly reduces the amount of soluble arsenic. In interpreting this he suggested that chemical action is lessened by the spreader which has enveloped the arsenate of lead or sulphur particle, as the case may be. In certain tests against the powdery mildew of hops Goodwin, Martin, and Salmon (20) obtained an increase in the effectiveness of sulphur with the addition of casein lime.

Kolotex 8–50 and Kayso (casein lime) ½–50, applied together as a spray after an infection period of 72 hours, were somewhat more effective than Kolotex used alone as a dust (Table 2, Ser. 36), although it is doubtful whether this difference is significant. The addition of Kayso ½–50 to lime sulphur 1–40 plus arsenate of lead 1–50 seemed to increase the effectiveness when applied 72 to 90 hours after inoculation began (Table 2, Ser. 29–31). Lime sulphur plus Kayso plus arsenate of lead appeared to be consistently superior in varying degrees to lime sulphur plus arsenate of lead and more effective than mixtures prepared in the following order: lime sulphur plus arsenate of lead plus Kayso and Kayso plus arsenate of lead plus lime sulphur.

Emulsified Oil[6]

The possible value of emulsified oil as an orchard spray has been investigated chiefly by entomologists. A noninjurious effective insecticidal and

[6] Investigations relative to some limiting factors in the use of saturated petroleum oils are reported by Knight, Chamberlin, and Samuels (31).

fungicidal emulsified oil for summer spraying is still in the developmental stage. Emulsified oil L202 seemed to be comparable with Bordeaux mixture and superior to certain sulphur dusts when applied 46 to 72 hours after inoculation (Table 2, Ser. 1, 3, 4). However, situations may arise in which emulsified oil L20 is ineffective after infection periods of from 24 to 30 hours (Table 2, Ser. 38–40). L205,[7] an emulsified oil containing a form of sulphur, applied 24 to 72 hours after inoculation, apparently did not offer such good control as the sprays containing lime sulphur but seemed to be more effective than oil emulsion alone, certain sulphur dusts, finely divided sulphur sprays, and Bordeaux mixture (Table 2, Ser. 1–4, 38–40). L205 may not be so toxic in some instances, even when applied a comparatively short time after inoculation (Table 2, Ser. 38–40). Kolodust 8–50 (Table 2, Ser. 1, 3, 37), Kolotex 8–50 (Table 2, Ser. 4, 36), and Bentonite sulphur 5–50 (Table 2, Ser. 38), applied in a 2 per cent emulsified oil, L202 or L20, after infection periods of 30 to 72 hours, were decidedly more efficient than when used alone as dusts or sprays. These combinations are not quite so effective in post-infection treatments as L205 but, as shown later, are more adhesive. K–1–CB, 117C, 117E, and 118, which are mercurial compounds, may be decidedly effective as fungicides in the 2 per cent oil emulsion L20, when applied 24 to 30 hours after inoculation (Table 2, Ser. 34, 38–40).

Soft Soap

Goodwin and Salmon (22) found that sulphur in suspension in a solution of soft soap gives more rapid and complete control of *Sphaerotheca humuli* than sulphur used as a dust. Goodwin, Martin, and Salmon (20) are of the opinion that soft soap not only gives improved dispersion but increases the fungicidal action of sulphur by virtue of its alkalinity, which hastens the hydrolysis of the sulphur.

Experiments were conducted in which Kolodust 8–50 (Table 2, Ser. 37), Kolotex 8–50 (Table 2, Ser. 36), and Bentonite sulphur 5–50 (Table 2, Ser. 38), respectively, were applied in a solution of 1 per cent soft soap, following infection periods of 48, 72, and 30 hours, respectively. The data show a distinct increase in effectiveness for this combination as compared with the sulphur dusts used alone. Soft soap is in the majority of cases more effective than oil emulsion in this particular kind of test. Neutral sodium and potassium soft soaps exhibit fungicidal properties (Table 2, Ser. 6, 16, 38).

RELATION OF THE DISTRIBUTION OF CERTAIN FUNGICIDES TO THEIR EFFECTIVENESS

The rate of growth of the host or the amount of unprotected susceptible foliage, flowers, and fruit throughout the season, under varying environ-

[7] L205 has been found to cause injury under certain conditions in the field.

mental conditions, is an important factor to be considered in spraying and dusting programs. Inasmuch as sulphur may be effective across space, the question arises as to the relation of degree of coverage of apple foliage to the amount of infection.

The fungicidal action of sulphur across space. That the germination of spores is inhibited even when they are not in direct contact with sulphur particles has been demonstrated frequently. In the progress report of Barker, Gimingham, and Wiltshire (2) it was shown that sulphur could exercise a definite toxic influence on a living fungus spore over considerable distances. Young (61) attributed the inhibition of germination of fungus spores to pentathionic acid, which he stated is volatile. Barker (3, 4) claimed to have proved conclusively that free sulphur from any of the ordinary forms of sulphur is dispersed through space in the form of minute solid particles and that this emanation is continuous under suitable moisture and temperature conditions. From further investigations Barker (4) reports that sulphuretted hydrogen is formed as the direct result of interaction between the sulphur and the host plant or the fungus at ordinary temperatures. Marsh (34), working in the same laboratory, concluded that this gas has considerable fungicidal action and that the quantity formed by living sulphured leaves was definitely toxic under the conditions of his experiments and might be a factor of importance in the greenhouse. Goodwin and Martin (24) claimed that sulphur vaporizes and condenses as minute solid particles.

TABLE 5.—*The effectiveness of Kolodust in controlling Wealthy apple leaf infection by the ascospores of Venturia inaequalis by fungicidal action across space*

Series and date	Inoc. chamber		Fungicide[a]	Trees inoc.	Final results average per tree	
	Temp.	Period in			Leaves infected	Lesions per leaf
	°C.	Hrs.		No.	No.	No.
1927						
Series 1[b]						
June 17	18	35	Untreated	3	2.7	41.5
"	18	35	Kolodust (8–50)	4	0.2	1.2
Series 2[b]						
June 19	23	24	Untreated	3	4.0	11.2
"	23	24	Kolodust (8–50)	4	0.5	3.0
1928						
Series 3[c]						
May 5	10	40	Untreated	3	12.3	17.6
"	10	40	Kolodust (8–50)	4	3.3	3.2

[a] Strips of cheesecloth dipped in a suspension of sulphur were hung about the trees during the infection period in the moist chamber. Care was taken to avoid any contact between these strips and the trees.

[b] Conidial inoculum of *Venturia inaequalis* from leaves in the orchard.

[c] Ascosporic inoculum of *Venturia inaequalis* from overwintered apple leaves.

TABLE 6.—*The relation of degree of coverage of Wealthy apple foliage to the effectiveness of certain fungicides against infection by the ascospores of Venturia inaequalis*

Series and date	Inoc. chamber		Fungicide[a]	Final results averaged per twig	
	Temp.	Period in		Leaves infected	Lesions per leaf
	°C.	Hrs.		No.	No.
1927					
Series 1[b]					
June 30	20	24	Untreated	4.2	56.0
"	20	24	L–S 1–40 + AL 1–50	0.3	1.5
"	20	24	S–Ars dust 90–10	1.0	5.0
Series 2[b]					
July 1	20	24	Untreated	4.0	70.6
"	20	24	L–S 1–40 + AL 1–50	0.0	0.0
"	20	24	S–Ars dust 90–10	0.3	2.0
Series 3[b, c]					
July 15	23	24	Untreated	3.0	20.3
"	23	24	L–S 1–40 + AL 1–50	0.3	0.5
"	23	24	S–Ars dust 90–10	0.3	1.3
Series 4[b, c]					
July 19	23	24	Untreated	3.0	14.7
"	23	24	L–S 1–40 + AL 1–50	0.8	1.8
"	23	24	S–Ars dust 90–10	1.0	4.3
1928					
Series 5[d]					
Mar. 22	24	24	Untreated	3.0	70.3
"	24	24	L–S 1–40 + AL 1–50	0.0	0.0
"	24	24	L–S 1–40 + AL 1–50[c]	1.0	3.0
"	24	24	Kolotex	1.0	2.3
"	24	24	Kolotex[c]	1.6	4.4
Series 6[d]					
May 3	20	40	Untreated	4.8	51.8
"	20	40	BM 4–4–50	0.8	0.9
"	20	40	Kolodust	0.5	0.2
Series 7[d]					
May 5	10	40	Untreated	4.1	32.2
"	10	40	BM 4–4–50	0.8	0.5
"	10	40	Kolodust	1.0	0.8
1929					
Series 8[d]					
Feb. 15	20	48	Untreated	3.7	69.7
"	20	48	Untreated[e]	3.0	7.0
"	20	48	BM 4–4–50[f]	3.0	1.2
"	20	48	BM 4–4–50[g]	0.3	0.7
"	20	48	Untreated[e]	3.0	21.0
"	20	48	Lime 4–50[f]	3.0	7.3
"	20	48	Lime 4–50[g]	4.0	53.5
"	20	48	Untreated[e]	3.0	10.3
"	20	48	L20 2%[f]	3.0	3.0
"	20	48	L20 2%[g]	0.5	3.0
"	20	48	AL 2–50[g]	2.5	45.0

TABLE 6—(*Continued*)

Series and date	Inoc. chamber		Fungicide[a]	Final results averaged per twig	
	Temp.	Period in		Leaves infected	Lesions per leaf
	°C.	Hrs.		No.	No.
Series 9[d]					
Mar. 9	15	40	Untreated	4.3	76.7
"	15	40	Untreated[e]	3.5	42.1
"	15	40	BM 4–4–50[f, h]	3.5	10.0
"	15	40	Untreated[e]	3.0	54.7
"	15	40	Stone lime 4–50[f]	3.0	45.2
Series 10[d]					
Apr. 2	15	45	Untreated	4.2	85.5
"	15	45	Untreated[e]	2.8	19.8
"	15	45	BM 4–4–50[f, h]	2.8	3.6
"	15	45	Untreated[e]	3.5	39.8
"	15	45	Stone lime 4–50[f]	3.5	33.4
"	15	45	Stone lime 4–50[g]	4.3	59.0
"	15	45	AL 1–50[g]	3.5	38.0

[a] L-S = Commercial liquid lime sulphur. AL = Commercial powdered arsenate of lead. BM = Bordeaux mixture. L20 = Emulsified oil. Three leaves were treated for each twig.

[b] The trees were inoculated with conidia of *Venturia inaequalis* obtained from leaves in the orchard.

[c] The treated areas were delimited by cocoa butter.

[d] The trees were inoculated with the ascospores of *Venturia inaequalis* from overwintered apple leaves.

[e] One-half of the treated leaves was left as a control.

[f] The treatments were applied to only one side of the leaf (e).

[g] The foliage was completely covered by treatments.

[h] Stone lime was used in preparation of Bordeaux mixture.

Trees previously inoculated with the imperfect or perfect stage of *Venturia inaequalis* were placed in the center of the moist chamber (Fig. 1), a structure having a capacity of 130.5 cu. ft. inside the curtains. Within this space pieces of cheesecloth (183 sq. ft. in all) were suspended from the framework on two sides of the trees but not touching them. This material previously had been dipped in a water suspension of Kolotex 8–50 and had taken up between 70 and 100 grams of the sulphur. Strips treated in this manner were used dry or wet. Considerable control of the fungus was obtained (Table 5). As is to be expected, however, some infection occurred. This may occur in any of the experiments, for invariably a certain number of spores are much more resistant than others or more advantageously placed for infection. This would be quite manifest in such a delicate test. Sulphur appears to have been toxic under these conditions with infection periods of 24 to 40 hours at temperatures ranging from 10° to 23° C. Although the plants were not incubated at these temperatures, it is not probable that with infection periods of this duration the "con-

densed'' particulate sulphur would be effective to such a degree after the trees were removed to the greenhouse.

The relation of degree of coverage of apple foliage to amount of infection. The fungicides were applied in about $\frac{1}{8}$ inch bands evenly spaced transversely across each leaf. The area covered varied with the size of the leaves, each leaf usually receiving two or three bands. In some instances areas about $\frac{1}{4}$ inch square were treated at suitable intervals on the leaf or arranged in such a manner as to give fairly large unprotected areas. The results appear in table 6. It is quite evident that sulphur-arsenate dust 90–10 (Ser. 1–4); Kolotex (Ser. 5); Kolodust (Ser. 6, 7); lime sulphur 1–40 plus arsenate of lead 1–50 (Ser. 1–5); and Bordeaux mixture (Ser. 6–10) gave good control when present in restricted areas at a temperature range of 10° to 24° C. Except in an occasional experiment, as in series 8, lime 4–50 (Ser. 8–10) did not exhibit any appreciable toxicity, regardless of whether the treatment was applied as bands or complete coverage. Hydrated and stone lime were equally ineffective when applied in these ways. The effectiveness of the Bordeaux mixture was not altered when stone lime was used instead of hydrated lime. The concentrations of the material tested in the bands, within limits, did not appear to influence the degree of toxicity of the treatments. L20, an emulsified oil, seemed markedly effective when applied to limited areas on the leaf surface (Ser. 8). Complete coverage with arsenate of lead did not appreciably inhibit infection (Ser. 8, 10). Good infection occurred on untreated leaves above and below the treated leaves in all the series (Table 6) and on untreated halves of leaves in those series where only one-half was treated (Ser. 8–10).

In an attempt to limit the possible spread of the sulphur preparations used incident to water movements on the leaf, a series of experiments was conducted in which the areas to which the fungicide was applied were delimited by means of cocoa butter. The efficiency of the sulphur fungicides was not appreciably decreased (Ser. 3–5). The data on this point are not fully conclusive, however, because the growing leaves in some cases cracked the cocoa butter before the trees were removed from the moist chamber.

Apparently temperature did not materially change the effectiveness of the sulphur fungicides within the range of 10° to 24° C. The spores of the imperfect and perfect stages of the fungus were affected similarly.

Inasmuch as it was found that sulphur was effective over a larger area than was actually treated, rapidly growing apple trees were dusted or sprayed and after a certain amount of growth had taken place were inoculated with *Venturia inaequalis.* In nearly every case treatments with the sulphur fungicides, such as Kolodust, Kolotex, and lime sulphur 1–40 plus arsenate of lead 1–50, protected even the smallest leaf measurable at the

TABLE 7.—*The relative efficiency of certain fungicides in protecting rapidly growing Wealthy apple leaves against infection by the ascospores of Venturia inaequalis*

Series and date	Fungicide[a]	Inoculation chamber			Leaf size when fung. app.	Leaf size at end of infection period	Lesions per leaf
		Date	Temp.	Period in			
			°C.	Hrs.	Mm.	Mm.	No.
1927							
Series 1[b]							
June 25	Kolodust	June 30	20	24	?	7 x 25	0
					?	17 x 40	13
					?	22 x 50	0
					6 x 20	27 x 60	0
					10 x 20	31 x 55	0
"	L-S 1–40 + AL 1–50	"	20	24	?	12 x 32	5
					?	20 x 42	10
					?	30 x 55	0
					15 x 30	42 x 60	0
					35 x 58	47 x 70	0
1928							
Series 2[c]							
Mar. 14	Kolotex	Mar. 19	5	24	?	9 x 20	0
					?	17 x 35	17
					?	24 x 48	0
					12 x 24	30 x 55	0
					24 x 43	40 x 73	0
"	L-S 1–40 + AL 1–50	"	5	24	?	12 x 28	3
					?	18 x 37	0
					13 x 30	30 x 60	0
					20 x 50	40 x 83	0
Series 3[c,d]							
May 21	BM 4–4–50	May 26	16	42	?	?	26
					?	14 x 34	62
					?	24 x 50	n

TABLE 7.—(*Continued*)

Series and date	Fungicide[a]	Inoculation chamber			Leaf size when fung. app.	Leaf size at end of infection period	Lesions per leaf
		Date	Temp.	Period in			
			°C.	Hrs.	Mm.	Mm.	No.
					?	28 x 62	n
					16 x 35	42 x 72	40
					25 x 55	53 x 95	60
"	Kolodust	"	16	42	?	?	34
					?	15 x 34	70
					?	20 x 50	13
					13 x 28	35 x 60	0
					15 x 35	40 x 70	0
"	Lime 4–50	"	16	42	?	?	34
					?	22 x 50	n
					30 x 55	35 x 65	n
					24 x 48	45 x 80	70
Series 4[c]							
Feb. 27	Untreated	Mar. 1	15	35	?	?	2
					?	12 x 32	42
					10 x 32	20 x 49	70
					18 x 37	27 x 52	33
"	BM 4–4–50	"	15	35	?	14 x 31	17
					10 x 30	18 x 44	6
					15 x 42	24 x 52	6
"	Lime 4–50	"	15	35	?	?	7
					?	13 x 40	36
					14 x 38	22 x 57	n
					21 x 50	30 x 68	n
"	L20 2%	"	15	35	?	?	3
					?	12 x 32	1
					10 x 32	20 x 49	12

TABLE 7.—(*Continued*)

Series and date	Fungicide[a]	Inoculation chamber			Leaf size when fung. app.	Leaf size at end of infection period	Lesions per leaf
		Date	Temp.	Period in			
			°C.	Hrs.	Mm.	Mm.	No.
					18 x 37	27 x 52	27
1929							
Series 5[c]							
Apr. 2	Untreated	Apr. 5	15	45	?	?	6
					?	?	9
					?	21 x 48	n
					12 x 32	28 x 58	n
					17 x 42	35 x 68	n
"	BM 4–4–50[e]	"	15	45	?	?	2
					?	22 x 50	56
					13 x 35	26 x 60	14
					20 x 51	41 x 76	3
"	Untreated	Apr. 7	15	45	?	?	23
					?	?	59
					?	?	52
					10 x 30	37 x 75	n
					16 x 40	42 x 84	14
"	BM 4–4–50[e]	"	15	45	?	?	27
					?	?	n
					?	?	n
					11 x 30	38 x 77	14
					20 x 43	50 x 90	0

[a] L-S = Commercial liquid lime-sulphur. AL = Commercial powdered arsenate of lead. BM = Bordeaux mixture. L20 = Emulsified oil.

[b] The trees were inoculated with the conidia of *Venturia inaequalis* from leaves in the orchard.

[c] The trees were inoculated with the ascospores of *Venturia inaequalis* from overwintered apple leaves.

[d] Measurements were not made until May 28.

[e] Stone lime was used in the preparation of the Bordeaux mixture.

time of application throughout its period of growth (Table 7, Ser. 1–3). Frequently, a leaf younger than the smallest one measured also was protected. The period between fungicidal treatment and inoculation was usually 4 or 5 days. The effectiveness of the sulphur fungicides did not seem to be influenced to any marked degree by temperature within the range of 5° to 20° C. Both the imperfect and perfect stages of the fungus were tested with like results. That the trees were susceptible was evidenced to some degree by the infection of the unprotected leaves and the excellent infection on control trees. Bordeaux mixture may be remarkably effective in similar applications but in less degree than the sulphur (Table 7, Ser. 3–5). The Bordeaux mixture was equally effective whether hydrated or stone lime was used. Hydrated and stone lime were not effective in experiments similar to those in which Bordeaux mixture was used (Table 7, Ser. 3, 4). Plants were treated, respectively, with hydrated and stone lime and Bordeaux mixture made from each and placed at a temperature of 28° C. with relative humidities of 50 and 75 per cent. The results were not materially different from those obtained when treated plants were placed in the greenhouse until inoculation. Oil emulsion L20 was somewhat less effective than Bordeaux under the same conditions (Table 7, Ser. 4). These results with Bordeaux mixture seem to have considerable theoretical and practical interest. However, a more detailed treatment of the questions they raise lies beyond the scope of the present investigations.

THE EFFECTIVENESS OF CERTAIN FUNGICIDES FOR INACTIVATING THE CONIDIAL INOCULUM IN THE CONTROL OF VENTURIA INAEQUALIS

Field observations by Keitt and Jones (29) and others show that the development of sufficient secondary inoculum, followed by the cooler weather and increased atmospheric moisture that may occur later in the season, can initiate late infection of fruit and more abundantly establish the fungus on the foliage. This increased leaf infection is an important factor in providing a greater source of inoculum in the following year (Wilson, 59). Applications of dusts and sprays have been recommended for some time to prevent late infection. In the present study inquiry has been made into the effectiveness of certain fungicides in inactivating the conidia that are present and preventing further production of conidia.

Fungicides applied to well-developed apple-scab lesions in orchard foliage were found to be not only toxic to the spores present but able to prevent further sporulation of the mycelia for a considerable length of time, or actually to kill the fungus in these lesions. The effectiveness of the fungicide apparently was influenced by the type of lesion, the degree of moisture, or by both moisture and temperature. The results presented in figure 4, together with other data, show that sulphur-arsenate dust 90–10,

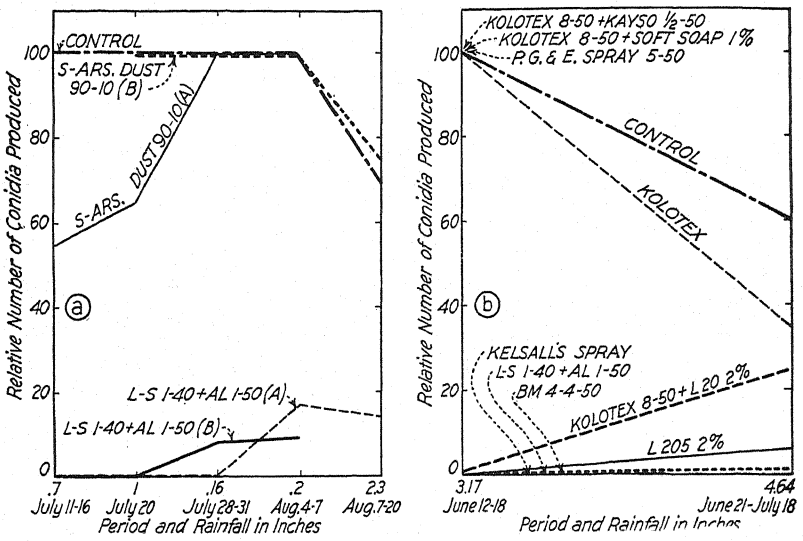


FIG. 4. Effectiveness of certain fungicides for inactivating the conidial inoculum in the control of *Venturia inaequalis*. The relative number of conidia produced on the untreated and treated leaves was determined about 3 days after each rain. Severely scabbed leaves were selected and thoroughly atomized to remove spores. Estimates of the number of spores in the washings were made by a standardized technique. Relative numbers were assigned, based on the control which is taken as 100. S-Ars. dust = Sulphur arsenate dust 90–10. L-S = Commercial liquid lime sulphur. AL = Commercial powdered arsenate of lead. Kayso = Casein lime. Soft soap = Neutral sodium soft soap (20th Century Soap). P. G. & E. spray = Prepared from a finely divided sulphur paste. Kelsall's spray = Lime sulphur-aluminum sulphate mixture. BM = Bordeaux mixture. L20 = Emulsified oil. L205 = L20 plus a form of sulphur.

applied to well-developed lesions did not inhibit production of conidia after 0.15 inch of rainfall and that there was little or no toxic effect from the treatment after 1 inch of rainfall. Kolotex and P. G. & E. sulphur paste 5–50, both of which contain finely divided sulphur, were apparently little more efficient than the coarser sulphur-arsenate dust 90–10. The addition of soft soap, 1 per cent, or Kayso ½–50 to Kolotex 8–50 did not seem to make the dust markedly more efficient, but the addition of 2 per cent of an emulsified oil, L20, gave increased effectiveness. The sprays Bordeaux mixture 4–4–50, lime sulphur-aluminum sulphate mixture, lime sulphur 1–40 plus arsenate of lead 1–50, and L205, 2 per cent, appeared appreciably to prevent the development of viable conidia even after 7.81 inches of rainfall, and, with the possible exception of the oil spray L205, they may be effective for the greater part of the season, depending on environmental factors. In addition, they may entirely kill the fungus in the lesions, but, as previously mentioned, this action is influenced by various factors. The

toxicity of lime sulphur plus arsenate of lead appreciably increased when the application was followed by rain or dews and a favorable temperature. Frequent rains together with other environmental factors have a marked effect on the quantity of conidia produced by nontreated lesions. From these data it appears that the secondary inoculum of *Venturia inaequalis* may be appreciably reduced according to the fungicidal effectiveness and adhesiveness of the treatments.

RELATION OF WASHING TREATMENTS TO THE EFFECTIVENESS OF CERTAIN FUNGICIDES[8]

In the study of the effectiveness of sprays and dusts one is confronted with the problem of duration of the period of protection per application. The data that have accumulated on this phase of the problem have been derived chiefly from three sources: laboratory studies, field studies, and chemical analyses of treated foliage. Laboratory studies may give comparative data but insufficient information as to what actually occurs in practice. Results of field investigations are often apparently contradictory, due, no doubt, to varying environmental conditions and other limiting factors. Chemical analyses of the treated foliage have given very valuable data but do not answer the question of whether or not foliage is adequately protected. The nature of the greenhouse facilities has enabled the writer to begin an investigation of this problem under partly controlled conditions. It was hoped that by treating the leaves of potted apple trees with various types of fungicides, subjecting them to a washing technique and finally inoculating them with ascospores of *Venturia inaequalis,* a more intimate knowledge could be obtained concerning the comparative effectiveness of materials in terms of disease control.

Apparatus and method. Potted Wealthy apple trees, as described on page 450, were used for these experiments. Specimens of equal or nearly equal height were selected for each experiment. At a suitable time after treatment the trees, with their tops at approximately the same level, were placed in a single row around the edge of a turntable situated in a moist chamber (Fig. 2, C). The turntable (Fig. 2, C) made 0.73 revolution per minute. A Skinner irrigation nozzle was placed about 5 feet above and a little to one side of the turntable. A rain gauge was placed between two of the trees, its aperture level with the top of the twigs, so as to obtain a record of the approximate amount of washing for the allotted time. The

[8] A literature review of spreaders and adhesives and a proposed theory of adherence will be found in the paper by Moore (38). Holland, Dunbar, and Gilligan (27) cite literature and give other data relative to wetting, spreading, and adhesiveness, and other properties of copper fungicides as influenced by the addition of various supplements.

TABLE 8.—*The relation of washing treatments to the effectiveness of certain fungicides in preventing infection of Wealthy apple leaves by the ascospores of Venturia inaequalis*

Series, date, and fungicide[a]	Washing treatment		Inoc. chamber		Final results averaged per twig			
	Amount	Time	Temp.	Period in	Leaves infected	Max. lesions on one leaf		Total lesions
	In.	Min.	°C.	Hrs.	No.	No.	Rel. No.	Rel. No.
Series 1[b], April 4, 1928								
Untreated	.....	.....	10	42	6.0	39.0	100	100
Kolotex	0.36	.....	10	42	0.0	0.0	0	0
"	0.50	.....	10	42	3.0	21.2	54	35
"	0.93	.....	10	42	1.5	6.9	18	9
"	1.08	.....	10	42	2.0	15.3	39	23
L-S 1–40 + AL 1–50	0.36	.....	10	42	0.0	0.0	0	0
"	0.50	.....	10	42	0.0	0.0	0	0
"	0.93	.....	10	42	0.0	0.0	0	0
"	1.08	.....	10	42	0.0	0.0	0	0
Series 2, May 16, 1928								
Untreated	2.00	60	20	40	3.0	22.3	100	100
" (not washed)	0.00	.....	20	40	1.6	3.3	15	12
Kolotex	2.00	60	20	40	0.8	1.5	7	4
"	4.00	120	20	40	3.0	25.3	113	99
Kolotex 8–50 + L20 2%	2.00	60	20	40	0.0	0.0	0	0
"	4.00	120	20	40	1.0	3.7	17	23
L205 2%	2.00	60	20	40	2.0	6.6	30	23
"	4.00	120	20	40	3.0	43.0	193	149
L-S 1–40 + AL 1–50	2.00	60	20	40	0.0	0.0	0	0
"	4.00	120	20	40	0.0	0.0	0	0
Series 3, May 19, 1928								
Untreated	1.00	25	15	42	4.8	97.8	100	100
Kolotex	1.00	25	15	42	3.0	69.3	71	69
"	2.00	50	15	42	5.3	90.0	92	87
Kolotex 8–50 + L20 2%	2.00	50	15	42	3.0	28.3	29	19
"	4.00	100	15	42	2.3	15.3	16	14
L-S 1–40 + AL 1–50	2.00	50	15	42	0.6	1.3	1	1
"	4.00	100	15	42	2.0	10.0	10	6
BM 4–4–50	2.00	50	15	42	3.6	7.3	7	7
"	4.00	100	15	42	3.6	44.0	45	42

TABLE 8.—(*Continued*)

Series, date, and fungicide[a]	Washing treatment		Inoc. chamber		Final results averaged per twig			
	Amount	Time	Temp.	Period in	Leaves infected	Max. lesions on one leaf		Total lesions
	In.	Min.	°C.	Hrs.	No.	No.	Rel. No.	Rel. No.
L205 2%	2.00	50	15	42	3.4	24.0	25	20
"	4.00	100	15	42	4.6	100.0	102	93
K-l-GB 2–50	2.00	50	15	42	5.5	90.0	92	135
"	4.00	100	15	42	5.5	100.0	102	193
Series 4[c], May 24, 1928								
Untreated	6.00	15	17	48	3.5	27.0	100	100
" (not washed)	0.00	.....	17	48	1.5	15.0	56	27
L-S 1–40 + AL 1–50	6.00	15	17	48	0.0	0.0	0	0
"	12.00	30	17	48	0.2	0.2	1	1
BM 4–4–50	6.00	15	17	48	2.4	6.8	25	17
"	12.00	30	17	48	1.7	1.7	6	7
L205 2%	6.00	15	17	48	4.0	37.0	137	109
"	12.00	30	17	48	4.0	46.7	173	154
Series 5, June 8, 1928								
Untreated	1.50	90	16	34	6.0	82.6	100	100
Kolodust	0.75	45	16	34	2.2	34.0	41	33
"	1.70	90	16	34	3.5	15.7	19	10
"	3.00	180	16	34	2.6	15.3	19	8
Kolodust 8–50 + soft soap 1%	1.50	90	16	34	2.7	20.7	25	13
"	3.00	180	16	34	3.0	29.3	35	17
Kolodust 8–50 + Kayso ½–50	1.50	90	16	34	3.2	16.7	20	12
"	3.00	180	16	34	3.0	29.7	36	20
Kolodust + Kayso 80–10	1.50	90	16	34	2.2	7.5	9	4
Kolodust 8–50 + L20 2%	1.50	90	16	34	0.4	2.6	3	1
"	3.00	180	16	34	1.2	2.5	3	2
Series 6, June 13, 1928								
Untreated	1.60	60	16	32	6.0	100.0	100	100
" (not washed)	0.00	.....	16	32	3.8	10.6	11	9
Kolodust	1.60	60	16	32	3.5	42.3	42	40
"	3.10	120	16	32	2.0	8.3	8	2
P. G. & E. sulphur paste	1.60	60	16	32	4.5	74.0	74	55
"	3.10	120	16	32	4.7	72.0	72	34

TABLE 8.—(*Continued*)

Series, date, and fungicide[a]	Washing treatment		Inoc. chamber		Final results averaged per twig			
	Amount	Time	Temp.	Period in	Leaves infected	Max. lesions on one leaf		Total lesions
	In.	Min.	°C.	Hrs.	No.	No.	Rel. No.	Rel. No.
Ferrox sulphur paste 5–50	1.60	60	16	32	2.0	13.0	13	6
"	3.10	120	16	32	2.3	10.0	10	5
L-S 1–40	3.10	110	16	32	2.0	2.0	2	1
"	6.00	230	16	32	1.0	5.3	5	2
Series 7, June 16, 1928								
Untreated	2.00	120	17	46	5.0	95.0	100	100
L-S 1–40 + AL 1–50	2.00	120	17	46	0.2	0.2	0	0
"	4.00	240	17	46	1.0	0.7	1	0
"	6.00	360	17	46	0.0	0.0	0	0
"	8.00	480	17	46	0.7	0.7	1	0
BM 4–4–50	2.00	120	17	46	1.0	2.2	2	1
"	4.00	240	17	46	3.5	11.2	12	8
"	6.00	360	17	46	3.0	22.5	24	1
"	8.00	480	17	46	2.7	8.6	9	4
C-L-Ars. dust 10–80–10	2.00	120	17	46	2.3	10.3	11	6
"	4.00	240	17	46	4.0	74.5	78	42
"	6.00	360	17	46	4.0	69.3	73	45
Series 8, June 2, 1928								
Untreated	1.50	90	21	30	4.7	74.7	100	100
Pomodust	1.50	70	21	30	5.0	86.0	115	99
"	3.75	180	21	30	4.7	70.0	94	94
Pomodust 8–50 + Fe_2O_3 1%	1.50	90	21	30	3.7	31.7	42	26
"	3.25	180	21	30	4.7	96.7	129	153
Pomodust + Fe_2O_3 80–20	1.50	90	21	30	3.2	19.5	26	20
"	3.25	180	21	30	4.2	61.2	82	81
Pomodust (coated with Fe_2O_3 2%)	1.50	90	21	30	2.8	8.2	11	11
"	3.25	180	21	30	5.5	61.2	82	113
Fe_2O_3 1%	2.00	90	21	30	5.2	100.0	134	171
Pomodust 8–50 + L20 2%	2.00	90	21	30	0.5	0.2	0	0
"	3.75	180	21	30	4.0	47.0	63	58

[a] L-S = Commercial liquid lime sulphur. AL = Commercial powdered arsenate of lead. BM = Bordeaux mixture. C-L-Ars. = Copper-lime-lead arsenate. L20 = Emulsified oil. L205 = Emulsified oil plus a form of sulphur. K-l-GB = Proprietary mercury compound. Soft soap = Neutral sodium soft soap. Kayso = Casein lime. Pomodust (coated with F_2O_3 2%) = Pomodust was suspended in a 2 per cent solution of ferric oxide, dried and resifted.

[b] The washing was by natural rain.

[c] The regular nozzles of the chambers were running full force.

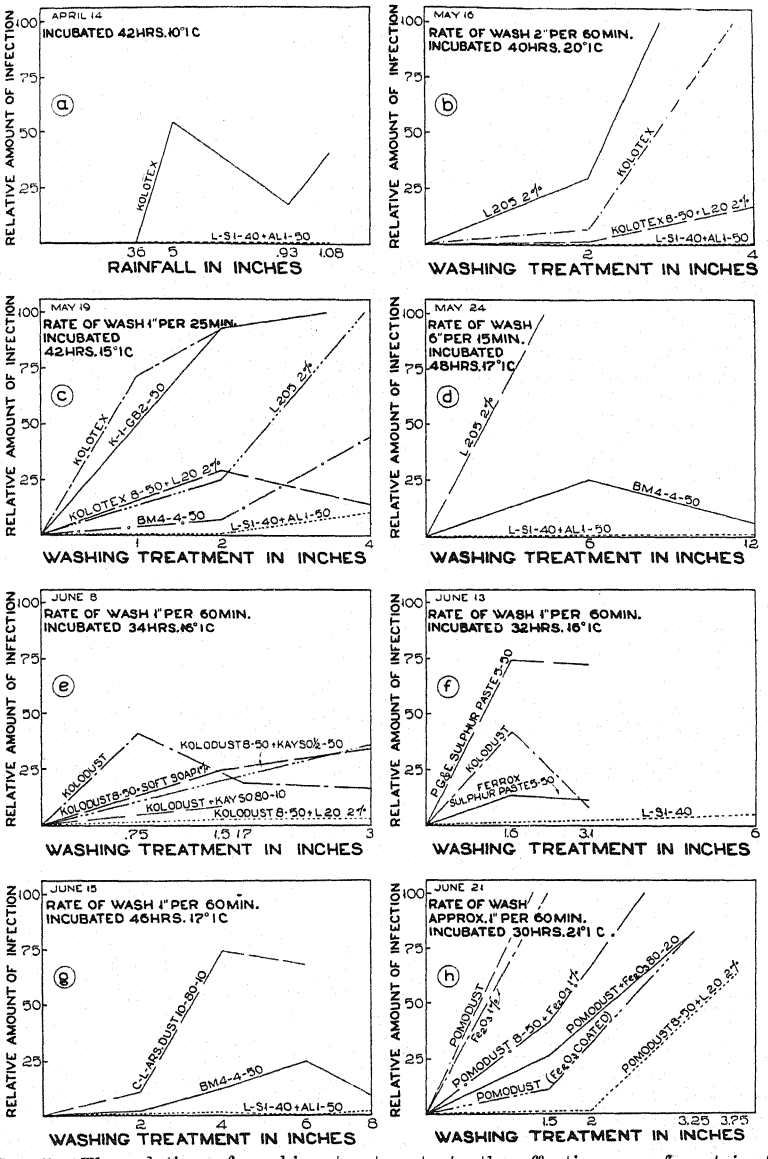


FIG. 5. The relation of washing treatments to the effectiveness of certain fungicides in preventing apple-leaf infection by the ascospores of *Venturia inaequalis*. L-S = Commercial liquid lime sulphur. AL = Commercial powdered arsenate of lead. L20 = Emulsified oil. L205 = L20 plus a form of sulphur. K-1-CB = Proprietary mercurial compound. BM = Bordeaux mixture. Kayso = Casein lime. Soft soap = Neutral sodium soft soap (20th Century Soap). C-L-Ars. = Copper-lime-lead arsenate. Pomodust (Fe_2O_5 coated) = Pomodust was suspended in a 2 per cent solution of ferric oxide, dried and resifted.

washing, in which lake water was used, was somewhat similar to that of a fine, gentle rain. After treatment the trees were inoculated in the usual way with the ascospores of *Venturia inaequalis* and then incubated in the greenhouse for a suitable time. The method of taking results was similar to that in previous experiments.

Treatments and results. Thatcher and Streeter (51) found that, after the first major mechanical loss, rain was apparently a minor factor in affecting the adherence of sulphur dust to foliage. Their data show a great mechanical loss for the sulphur dust during the first week, and by the end of the second week the major part of the sulphur, even in heavy dustings, was removed. Subsequent decrease in the amount of sulphur was small and rather uniform during the ensuing weeks. In the use of lime-sulphur spray the proportion of sulphur washed off during the early period was much less than that for the dust, and the rate was more gradual, especially for the first three weeks.

It was reported by Young and Tisdale (63) that particulate sulphur stuck better than coarse particles and that a heavy downpour of rain washed off less sulphur than the same amount of water falling more slowly and over a longer period.

A summary of treatments and of results appears in table 8 and figure 5. Sulphur dust was much inferior in adhesiveness to sulphur deposited by sprays containing lime sulphur (Table 8, Ser. 1, 2, 3, 6, or Fig. 4, a, b, c, f). Apparently, after a light rain or a short washing period, the major part of the sulphur dust was washed off, for under these conditions considerable infection took place. In the case of sprays containing lime sulphur enough of the sulphur adhered to the foliage after the so-called first major mechanical loss to provide protection even after a heavy rain or prolonged washing treatments. Bordeaux mixture was slightly less effective than lime sulphur after prolonged washing (Table 8, Ser. 4, 7, or Fig. 5, d, g). Copper-lime-arsenate dust was decidedly less adhesive than Bordeaux (Table 8, Ser. 7, or Fig. 4, g). P. G. & E. sulphur paste and Ferrox sulphur paste, both finely divided wettable sulphur products, were much inferior to lime sulphur in their efficiency for scab control after washing treatments (Table 8, Ser. 6, or Fig. 4, f). A proprietary emulsified oil, L205, containing a fungicide, was not significantly more effective than sulphur dust (Table 8, Ser. 2, 3, 4, or Fig. 5, b, c, d). It appears that the toxic agent was washed out, since oil emulsion to which sulphur dust was added at the time of application was decidedly more effective, as will be shown later. Perhaps this was due to the greater amount of sulphur in the latter mixture. A proprietary mercury compound did not appear to withstand washing any better than Kolotex (Table 8, Ser. 3, or Fig. 5, c). Arsenate of lead did

not appreciably increase the effectiveness of sulphur dust under the conditions of these experiments.

Casein lime may increase to a small degree the durability of the sulphur dust when proportioned for use either as a spray or dust (Table 8, Ser. 5, or Fig. 5, e). Soft soap was no more satisfactory than Kayso in rendering the sulphur dust more effective (Table 8, Ser. 5, or Fig. 5, e). The oil emulsion, L20, was the most promising of the stickers or spreaders tested with the sulphur dusts under these conditions (Table 8, Ser. 2, 3, 5, 8, or Fig. 5, b, c, e, h). The results (Table 8, Ser. 8, or Fig. 5, h) are in accord with the findings of Ginsburg (14, 16). Increased adhesiveness was manifest whether ferric oxide was added directly to sulphur dust, which may be proportioned for use either as a spray or dust, or was used as a coating on the sulphur-dust particles.

Control trees which had not been given a special washing treatment just before inoculation offered marked inhibition to the fungus (Table 8, Ser. 2, 4, 6). These trees, along with all the other uninoculated trees, had been well washed with a spray of water at frequent intervals with the aim of keeping the foliage clean. The variation in durability of the same fungicides for different series is due, in part, to a difference in the height of the twigs of the trees, the position of the trees on the table, the angle of the nozzle, the rate of washing, and the type of foliage. It must be kept in mind that these data are fragmentary. However, it is thought that considerable value attaches to the comparison of the various treatments within a series and that the data, taken as a whole, are significant regarding certain aspects of the relative adhesiveness of the fungicides tested.

RELATION OF THE ADDITION OF POTASSIUM PERMANGANATE TO THE TOXICITY OF SULPHUR DUST

Young's theory that the fungicidal effect of sulphur is due to the formation of pentathionic acid by oxidation in the presence of oxygen and water led Lee and Martin (32) to think that the fungicidal effectiveness of sulphur might be increased by adding oxidizing agents. They made field experiments on the control of eyespot disease of sugar cane, from which they concluded that the addition of 1 per cent potassium permanganate to sulphur dust increased its efficiency 200 to 300 per cent. The present writer, upon conducting comparative tests of various brands of sulphur acting across space, failed to find that the presence of 1 per cent potassium permanganate in the sulphur increased the effectiveness of the sulphur to any great extent. This indication is congruent with the more recent work of Bailey and Greaney (1), who conducted an investigation in connection with the control of leaf and stem rust of wheat. Goodwin and Martin (24) give data to indicate the nonproduction of a volatile sulphur derivative of

TABLE 9.—*The relation of the addition of potassium permanganate to the toxicity of sulphur dust to the spores of Sclerotinia cinerea in van Tieghem cells*[a]

Fungicide	Position of fungicide	Incubator		Final results		
		Temp.	Period in	Germination	Length of germ tubes	
		°C.	Hrs.	Per cent	Microns	Rel. No.
Series 1						
Control		20	8	90.7	82.8	100
Super-sulfodust	Bottom of cell	20	8	97.2	78.9	95
Super-sulfodust + $KMnO_4$ 1%	"	20	8	93.3	66.8	81
Series 2[b]						
Control		28	10	100.0	123.0	100
Super-sulfodust	Bottom of cell	28	10	35.4	12.3	10
Super-sulfodust + $KMnO_4$ 1%	"	28	10	30.0	8.2	7
$KMnO_4$	"	28	10	30.0	123.0	100
Series 3						
Control		28	8	95.3	75.0	100
Super-sulfodust	Bottom of cell	28	8	73.3	29.9	40
Super-sulfodust + $KMnO_4$ 1%	"	28	8	86.0	30.7	41
Series 4[c]						
Control		20	8	98.0	81.2	100
Super-sulfodust 10^2	In spore drop	20	8	0.0	0.0	0
Super-sulfodust 10^3	"	20	8	65.6	22.6	28
Super-sulfodust 10^4	"	20	8	92.0	53.3	66
Super-sulfodust + $KMnO_4$ 1% 10^2	"	20	8	6.0	12.3	15
Super-sulfodust + $KMnO_4$ 1% 10^3	"	20	8	71.0	25.8	32
Super-sulfodust + $KMnO_4$ 1% 10^4	"	20	8	59.0	41.0	50
$KMnO_4$ 10^3	"	20	8	0.0	0.0	0
$KMnO_4$ 10^4	"	20	8	66.1	45.1	56
Series 5[b]						
Control		20	8	98.0	109.5	100
Oxidized sulphur	Bottom of cell	20	8	48.0	32.8	30
Ferrox sulphur dust	"	20	8	63.1	44.3	40
Super-sulfodust	"	20	8	51.0	22.9	21

[a] The technique is described under the section on laboratory studies.

[b] Excess of the fungicides was used in the bottom of each cell. Oxidized sulphur = Sublimed sulphur plus potassium permanganate and a catalyst.

[c] The concentration of fungicide is denoted by 10^2, 10^3, 10^4. Growth developed later in the spore drops containing $KMnO_4$.

acid reaction from the use of potassium permanganate with sulphur in the presence of heat and moisture. Williams and Young (58) reported that sulphur, treated with certain oxidizing agents, such as potassium permanganate, was extremely toxic and was effective in the control of apple scab. Martin (35) obtained data which further substantiate the results of Lee and Martin (32).

Laboratory studies. The use of potassium permanganate with sulphur was studied in the laboratory, employing the technique described in a later section. The data obtained are presented in table 9, and figure 6, a, b. Percentage germination and reduction in length of germ tube of the conidia of *Sclerotinia cinerea* were used as criteria of toxicity. Certain tests were conducted across space in van Tieghem cells at 20° to 28° C. (Table 9, Ser. 1, 2, 3, 5, and Fig. 6, b). The fungicides were placed at the bottom of the cell, and the spores, in the hanging drop. The data do not suggest any significant increase in the effectiveness of Super-sulfodust or sublimed sulphur through the addition of potassium permanganate under these conditions. The trend of the results does not appear to be altered materially by a rise in temperature from 20° to 28° C. or by the amount of mixture used. Potassium permanganate in itself was not active across space. Combinations similar to the above were tested in the hanging drop itself at 20° C. (Table 9, Ser. 4, Fig. 6, a). The data corroborate the above results. Potassium permanganate was found to be more effective than sulphur when used alone in the hanging drop at the same concentration as with the sulphur (Table 9, Ser. 4).

Greenhouse studies. When oxidized and sublimed sulphur were compared in treatments made after infection periods of from 45 to 65 hours (Table 2, Ser. 1, 2, 4), there was no evidence that the oxidized sulphur was substantially more effective than sublimed sulphur. A comparison of oxidized sulphur with other types of dusts and sprays applied after infection periods of from 48 to 64 hours did not reveal any unusual effectiveness of the oxidized product (Table 2, Ser. 5, 37). Temperature within the range of 10° to 23° C. and a moist treatment after application appeared to play a rather insignificant rôle in increasing the amount of control with the activated sulphur. Potassium permanganate, 2 per cent, gave excellent control as compared with the other treatments listed, when applications were made 45 to 66 hours after inoculation began (Table 2, Ser. 1, 4).

SOME OBSERVATIONS ON THE EFFECTIVENESS OF FUNGICIDES

In analyzing the data relative to the control of *Venturia inaequalis* by means of various fungicidal treatments one must consider certain related factors, some of which have not been mentioned. It is quite possible that

there is a variation in the degree to which the ascospores of *V. inaequalis* can withstand the action of toxic materials. This may possibly be influenced by such factors as the "strains" of fungus used, the time of the year at which the spores matured, the vigor of individual spores, and the abundance of inoculum. The length of time after inoculation that control may be obtained is undoubtedly somewhat variable due to the time required for the leaves to collect sufficient moisture[9] for germination, and other factors. It is quite obvious that the trees vary in resistance at different times. Usually the three or four leaves most recently unfolded are susceptible to infection on the ventral surface, but, occasionally, trees will be found on which practically all the foliage is susceptible. Frequently it is found, especially where a fungicide was not applied, that the fungus in lesions on the more susceptible leaves at the top of the twigs breaks through the cuticle and sporulates, whereas, farther down the twigs the leaves are more mature and sufficiently resistant to prevent macroscopic development of lesions.

The trees grown in the greenhouse showed evidence of considerable variation in the carbohydrate-nitrogen ratio as conditions changed during the course of the season. The nitrogen-high condition seemed to increase the susceptibility to scab infection and to lime-sulphur injury. The nitrogen-high condition which commonly developed in the plants first forced (February and March) was corrected by providing artificial light.

In the case of post-inoculation treatments, especially in the use of the more effective sprays such as lime sulphur, it was found that the development of macroscopic lesions was delayed for days and the fungus in them might never fruit. Although this may not have any effect on the development of the perfect stage of the fungus, it checks the development of the conidial inoculum and in this way is a factor in fungicidal effectiveness which is not fully apparent from the data.

As a result of the rapid growth of apple trees in a shaded greenhouse, the texture of the leaves is somewhat different from that of the leaves of trees grown in an unshaded greenhouse. The trees used in these experiments were shaded for the most part during the periods of excessive heat so that infection would take place and lesions would appear.

LABORATORY STUDIES: APPARATUS AND METHODS

The conidia and ascospores of *Venturia inaequalis* and the conidia of *Sclerotinia cinerea* were used in laboratory studies. The conidia of *V.*

[9] The amount of particulate water that passed through the cloth top of tenting material over the iron frame in the moist chamber varied somewhat in the earlier work. This variation was eliminated on March 12, 1929, and, thereafter, by spraying the top from within just before the experiment began. This procedure filled the small apertures in the fabric with water, prevented the passage of droplets, and insured a saturated atmosphere without the danger of excess water.

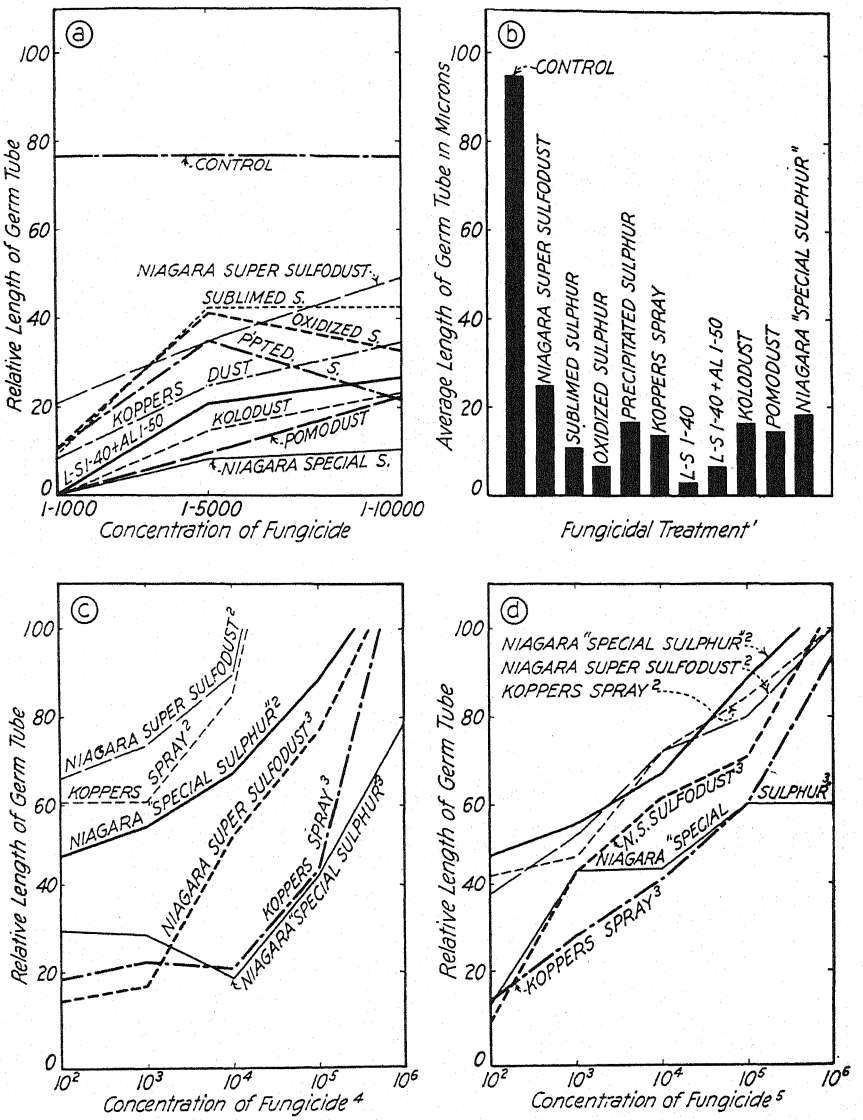


FIG. 6. The toxicity of certain sulphur fungicides to the conidia of *Sclerotinia cinerea* as determined by inhibition in length of germ tube in van Tieghem cells. Relative length of germ tube is based on the control which is taken as 100. Oxidized S = Sublimed sulphur plus potassium permanganate, 1 per cent, and a catalyst. P'pted S = Prepared by aerating lime sulphur. Koppers dust = Prepared from Ferrox flotation sulphur paste. L-S = Commercial liquid lime sulphur. AL = Commercial powdered arsenate of lead. Niagara "special sulphur" = Bentonite sulphur. Koppers spray = Prepared from Ferrox flotation sulphur paste. a. Fungicide and conidia in hanging drop, 20° C. b, c, d. Fungicide in bottom of cell, 28°, 17°, and 17° C., respectively. 1. A 2 per cent suspension of the fungicide was used. Aliquots of the suspension to be tested were dried down and slightly moistened just before the experiment was begun. 2. Conidia of *Sclerotinia cinerea*. 3. Conidia of *Venturia inaequalis*. 4. Sulphur dry. (Aliquots of the suspension to be tested were dried down.) 5. Sulphur wet. (Aliquots of the suspension to be tested were not dried down.)

inaequalis were washed from apple leaves from the orchard by means of an atomizer. In order to secure a clean leaf surface and a fresh crop of spores, the leaves were washed with sterile distilled water 3 or 4 days before they were collected, and covered with parchment bags. The ascospores of *V. inaequalis* were ejected from perithecia in moistened apple leaves into sterile conductivity water. *Sclerotinia cinerea* was cultured on potato-dextrose agar at 20° C. and the spores were taken from 7- to 9-day-old cultures.

The hanging-drop technique was used. Conductivity water was used for spore suspensions and the preparation of fungicides. The glassware was cleaned with potassium bichromate and sulphuric acid cleaning solution, thoroughly rinsed in ordinary distilled water, then in sterile conductivity water, and finally wiped with clean cheesecloth. All cover slips and slides were flamed just before using. The rings were 10 x 18 mm. The cells, unless otherwise stated, were left unsealed with a small opening at the edge of the cover slip to allow free access of oxygen. Four cells were used for each dilution. The drops of the spore suspension to be used as an indicator of the toxicity of the fungicide were taken with a platinum loop 2.5 mm. in diameter by a standardized procedure. The drops contained about 15 spores per low-power field of the microscope, or approximately 100 to 200 spores per drop.

The dilutions of the fungicide were based on the dry weight of the sulphur or sulphur mixture, except that, in the case of sprays containing lime sulphur, the dilution 1–40 was used as a basis. Aliquots of the various dilutions to be tested across space were transferred by means of a platinum loop 6.5 mm. in diameter. One loopful of a 1 per cent suspension of the sulphur preparation to be tested was placed in the bottom of each cell, unless otherwise stated. These drops were allowed to evaporate slowly until air-dry and then slightly moistened before the cover slip bearing the spores in a drop of sterile conductivity water was suspended over them. When the fungicidal dilutions were tested in the spore suspension, its evaporation was prevented by placing a large loop of water in the bottom of the cell. Water was used instead of the same fungicide that was in the hanging drop to avoid the complication of having any fungicide at the bottom of the cell where it might act across space on the spores at the bottom of the hanging drop. The cells were then incubated in sterile Petri dishes freshly lined with moist filter paper. Readings of germ-tube length and percentage of spores germinated were made at suitable times. The results of this work showed that the percentage of spores germinated was not a satisfactory criterion of the toxicity of the materials tested. Ordinarily 100 germ tubes were measured for each dilution. At the end of the incubation period the spore drops were allowed to evaporate to dryness and the cover

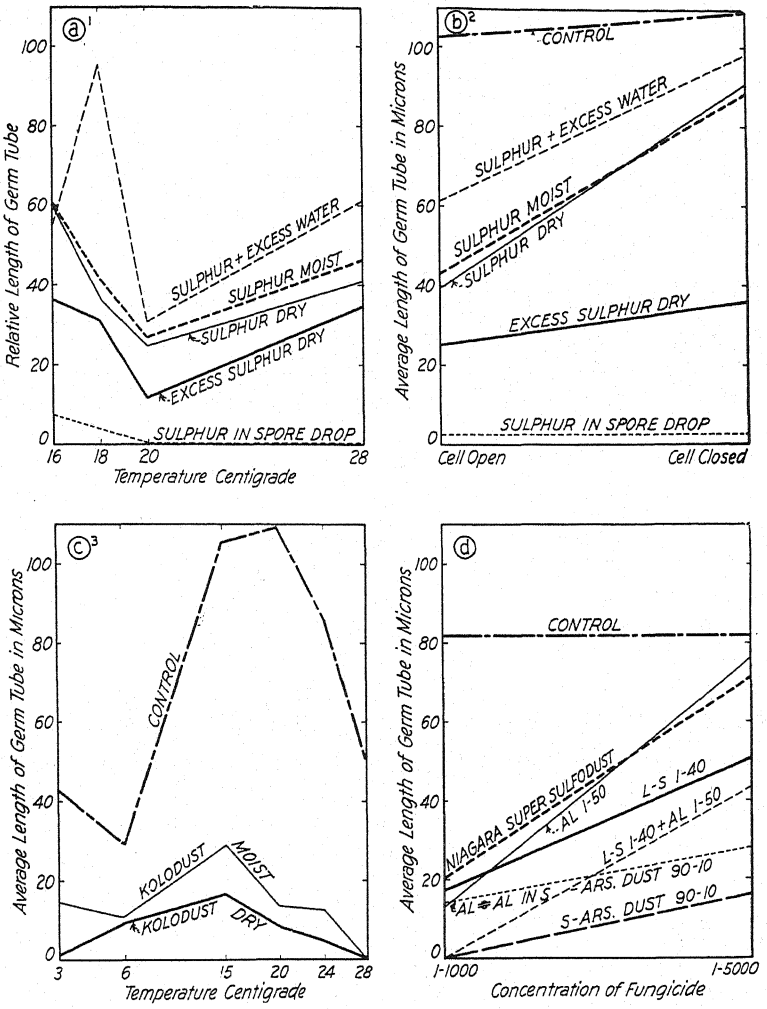


FIG. 7. Relation of water, temperature, oxygen, and arsenate of lead to the toxicity of certain fungicides as determined by inhibition in length of germ tube in van Tieghem cells. Relative length of germ tube is based on the control which is taken as 100. a, b. Conidia of *Sclerotinia cinerea*. Fungicide in bottom of cell. c. Ascospores of *Venturia inaequalis*. Fungicide in bottom of cell. d. Fungicide and conidia of *Sclerotinia* cinerea in hanging drop, 20° C. 1. Sulphur = Niagara Super-sulfodust. Sulphur + excess water = Aliquots of the suspension to be tested were not dried down. Sulphur moist = Aliquots of the suspension to be tested were dried down and slightly moistened just before experiment was begun. Each temperature is average of 2 series. 2. Sulphur = Niagara Super-sulfodust. The data are based on the average of 8 series, 2, each, for the temperatures 16°, 18°, 20° and 28° C. The degree of inhibition in the effectiveness of the sulphur due to the closed cell was not significantly different at any one of these temperatures. 3. A 2 per cent suspension of the dust was used.

slips dipped in formal-acetic-alcohol and stored in water until readings could be made. Any deviation from the technique as described in this section is noted in each particular instance.

No readings were taken from those spore drops that were clearly abnormal, as in the following cases. Under certain conditions germ tubes grew up into the drop away from the lower surface, thus escaping full exposure to the toxic agent; spores germinated and grew more readily in clumps than when evenly dispersed throughout the drop; irregularity in the size of drop due to the effect of surface tension between the drop and the glass surface sometimes caused variation with regard to the distribution of the spores; if the cells were not level, the spores would collect on the edge of the drop, thereby producing an abnormal oxygen relationship; and considerable variation in the number of spores per drop occasioned a variance in the effectiveness of the material used.

The technique used for making the sulphur suspensions was standardized as far as possible.

A COMPARISON OF THE TOXICITY OF CERTAIN SULPHUR FUNGICIDES AT VARIOUS CONCENTRATIONS IN THE SPORE DROP

That the fineness of the sulphur particle is a fungicidal determinant has been reported by various workers since the early investigation of fungicides, as reviewed by Windisch (60). This property of sulphur, which is considered important in relation to its adhesiveness and toxicity, has recently received considerable attention by Young (61) and Tisdale (52). Goodwin, Martin, and Salmon (20) found that colloidal sulphur was apparently decidedly more effective than wetted ground sulphur as a spray with soft soap against the hop "powdery mildew" (*Sphaerotheca humuli*).

The data (Fig. 5, a) show on a comparative basis the relative toxicity of various sulphur dusts when tested in the hanging drop, using the conidia of *Sclerotinia cinerea* as a criterion of toxicity at 20° C. It appears that any significant difference in the effectiveness of the several materials tended to disappear as the concentration in the spore drop increased below 1–1000.

Across space

The fact that sulphur acts across space is reported by authorities already cited (Barker, Gimingham, and Wiltshire (2), Young (61), Barker (3, 4), Goodwin and Martin (24), Marsh (34)). Figure 6, b, c, d, and figure 7, a, b, c, show the effectiveness of sulphur fungicides across space in van Tieghem cells. Figure 6, b, c, d, is representative of the comparative data obtained relative to several sulphur fungicides, the toxicity being measured by the inhibition in length of germ tube of the conidia of *Sclerotina cinerea* and *Venturia inaequalis* at 17° and 28° C. It is apparent that there was no

significant difference in any of the sulphur materials tested under these conditions, although it would appear from figure 6, c, d, that Niagara Super-sulfodust was less effective than the more finely divided Niagara "special sulphur" (Bentonite sulphur), except, perhaps, in the presence of water. The materials tested include Niagara Super-sulfodust, Pomodust, Kolodust, Niagara "special sulphur" (Bentonite sulphur), Koppers spray (Ferrox sulphur paste), precipitated sulphur (prepared by aerating lime sulphur), sublimed sulphur, oxidized sulphur, lime sulphur, and lime sulphur plus arsenate of lead.

THE EFFECT OF TEMPERATURE AND WATER ON THE TOXICITY OF CERTAIN SULPHUR FUNGICIDES

Studies made in the laboratory on the effect of temperature and humidity or water on the amount of control obtained with some sulphur fungicides by Doran (10), Young (61), Tisdale (52), and Goodwin and Martin (24, 25) have been reviewed earlier.

Tests of the effect of temperature on the fungicidal action of sulphur across space were conducted in van Tieghem cells in which the sulphur (Super-sulfodust) was placed at the bottom of the cell and a conidial suspension of *Sclerotinia cinerea* or an ascosporic suspension of *Venturia inaequalis* was placed above as a criterion of toxicity. The greatest inhibitory action of sulphur across space against the conidia of *S. cinerea* and the ascospores of *V. inaequalis* seemed to be about 20° C. under these conditions (Fig. 7, a, c). An interpretation of these results should take into account the effect of temperature on the germinating spore, as well as on the toxicity of the sulphur. Sulphur apparently was toxic to the ascospores of *V. inaequalis* when tested across space at 3° C. (Fig. 7, c).

Water may decrease the inhibitory action of sulphur across space, and this effect does not appear to be influenced more at one degree of temperature than at another within the range of 3° to 28° C. (Fig. 6, c, d, and Fig. 7, a, b, c). Droplets of lime sulphur 1–40, air dried at the bottom of the cell and then covered with a drop of water, showed no toxic effect across space on the conidia of *Sclerotinia cinerea.*

The data (Fig. 7, b) show that there was a distinct decrease in the amount of inhibition obtained with sulphur when tested across space on the conidia of *Sclerotinia cinerea* in sealed cells, as compared with that obtained in open cells. This effect was not so marked in cells in which sulphur was used in excess in the bottom of cell or in the spore drop. Young (61) reported that there was not sufficient oxygen in sealed cells to permit optimum effectiveness of sulphur. Goodwin and Martin (24) state that oxygen does not appear to be of importance in the production of the "volatile sulphur derivative" from sulphur which produces a stain on copper foil.

THE FUNGICIDAL ACTION OF ARSENATE OF LEAD

Laboratory tests which have been adequately reviewed by Butler and Doran (18) indicate that arsenate of lead is not lethal to *Venturia inaequalis* at the strength used in orchard spraying but that it increases the toxicity of lime sulphur.

Arsenate of lead was found markedly to increase the toxicity of Sulfodust and lime sulphur against the conidia of *Sclerotinia cinerea* in the hanging drop at 20° and 17° C., respectively, and to have fungicidal properties of its own (Fig. 6, a, and Fig. 7, d). Other data indicate that soluble arsenic is fungicidal and apparently inhibitory in its action. Inhibition is evident over a wide range of dilutons.

SUMMARY

1. This study of the control of apple scab with fungicides has consisted chiefly of greenhouse experiments in which the fungicides could be applied at will to trees on which the disease was induced by inoculation under partly controlled conditions. Attention has been directed as far as possible toward a simulation of natural conditions. Special attention has been given to a study of the comparative efficiency of various sulphur fungicides and to the relation of certain factors of the environment to their effectiveness.

2. All fungicides applied before inoculation gave excellent control if the trees were not subjected to washing. These included a series of sulphur dusts, sprays containing lime sulphur, Bordeaux mixture, and certain emulsified oils. Leaves treated with sulphur dust or lime sulphur spray were efficiently protected until they became resistant. Bordeaux mixture was somewhat less effective. The fungicidal effectiveness of sulphur dusts and lime sulphur was not appreciably changed when applied to the foliage and given a period in the moist chamber before inoculation. Temperature within the range of 6° to 23° C. during an infection period appeared to be more important in its relationship to the development of host and parasite than to the toxicity of sulphur. Excellent control was obtained at 6° C.

3. The fungicides showed differences in effectiveness when applied after inoculation. Sprays containing lime sulphur appeared to be distinctly more effective than the sulphur dusts (whether finely ground, sublimed, "activated," or containing arsenate of lead), wettable sulphur sprays, calcium monosulphide, Bordeaux mixture, emulsified oils with or without a sulphur fungicide, and certain proprietary mercury compounds. Sulphur-arsenate dust 90–10 suggested little indication of offering control in certain tests when applied later than about 12 hours after inoculation, unless the application was followed by a moist treatment, while sprays containing lime sulphur, especially lime sulphur 1–40 plus arsenate of lead 1–50, gave

good control when the application was made after infection periods ranging from 30 to 72 hours, and in some cases longer. Under the conditions of these experiments the data do not seem to indicate any significant difference in scab control between the various wettable sulphur pastes or finely ground sulphur preparations and the less finely divided sulphur products tested, which is attributable to their various physical states. Bordeaux mixture gave consistent control and was more effective than certain sulphur dusts or finely divided sulphur sprays. Certain emulsified oil treatments and proprietary mercury compounds appeared to exhibit marked effectiveness. The quantity of ascosporic inoculum was a factor in the effectiveness of sulphur fungicides applied after inoculation. Fungicidal applications made after infection show that temperatures ranging from 6° to 23° C. during an infection period did not materially affect the control of *Venturia inaequalis,* while temperatures above 26° C. became an appreciable factor in the inhibition of the fungus. The degree to which temperature was a factor was determined by the length of time that elapsed after inoculation before exposure to the higher temperature, the length of exposure to that temperature, the temperature itself, and probably the relative humidity. It is apparent that the higher temperatures were most effective against the organism before penetration had taken place. Data have been obtained which show that moisture was an important factor in the maximum effectiveness of many fungicides, such as sprays containing lime sulphur, when applied after an infection period, in the control of *V. inaequalis.* Histological evidence shows that *V. inaequalis* may be effectively controlled after infection has taken place.

4. There is indication that lime sulphur was quickly effective when applied at the time of inoculation or shortly after but that it required considerable length of time to be effective if the treatment was made some time after inoculation.

5. Rapid drying of lime sulphur appeared to increase its effectiveness in applications made after inouculation.

6. Lime 3–50 was not found to diminish the action of lime sulphur plus arsenate of lead to any appreciable extent when applied after infection periods.

7. The effectiveness of sulphur-lead arsenate dusts or sprays applied after infection periods appeared to be increased if a moist treatment was given for a period following the application. There is evidence that the increased effectiveness of lime sulphur from the addition of arsenate of lead is due, at least in part, to soluble arsenic. Soluble arsenic, As_2O_5, may be fungicidal alone. Injury did not result, under the conditions of these experiments, when soluble arsenic, As_2O_5, was applied alone at the

concentration of 0.04 per cent or with lime sulphur 1–40 at the concentration of 0.03 per cent.

8. Kayso (casein lime) sulphur appeared to increase the fungicidal property of lime sulphur plus arsenate of lead when applied after infection but the effect was not striking. Lime sulphur plus Kayso plus arsenate of lead seemed to be the most effective combination tried.

9. The effectiveness of certain sulphur fungicides was found to be increased when applied in a soft-soap solution.

10. Oil emulsion applied before or after inoculation seemed to exhibit fungicidal properties. The addition of oil emulsion appeared markedly to increase the effectiveness of some fungicides.

11. The proprietary mercurial compounds tested may be decidedly effective, under certain conditions, when applied after infection.

12. Development of conidia of *Venturia inaequalis* on leaf lesions was checked according to the fungicidal effectiveness and adhesiveness of the materials used.

13. Certain sulphur fungicides and Bordeaux mixture exhibited effectiveness over a larger area than the actual surface covered.

14. No significant increase in fungicidal efficiency of sulphur followed the addition of potassium permanganate to sulphur as an oxidizing agent.

15. The sprays containing lime sulphur and Bordeaux mixture are apparently decidedly more adhesive than certain sulphur dusts, finely divided sulphur sprays, emulsified oils, and other fungicides tested. Sulphur dust was ineffective after a short period of rain or washing treatment, while the lime-sulphur-containing spray was protective after a heavy rain or prolonged washing treatment.

16. The addition of such spreaders or adhesives as soft soap, emulsified oil, Kayso (casein lime), and ferric oxide increased the adhesivenes of certain fungicides to a greater or lesser extent. Emulsified oil gave the most satisfactory results.

17. Laboratory studies corroborated the greenhouse studies of sulphur fungicides as regards the inhibitory action of sulphur, the action of sulphur other than by contact, the effectiveness of potassium permanganate as a fungicide and its ineffectiveness as an "oxidizer" for sulphur dust, the fungicidal effectiveness of precipitated (aerated) lime sulphur, certain temperature relationships, and the fungicidal rôle of arsenate of lead and soluble arsenic.

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RELATIVE SUSCEPTIBILITY OF VARIETIES OF SORGHUM TO RUST, PUCCINIA PURPUREA[1]

C. O. JOHNSTON AND E. B. MAINS

INTRODUCTION

The many varieties of sorghum which are grown in the United States as grain, forage, or sirup crops, under many different environmental conditions, are known to be attacked by several fungous and bacterial diseases. In the literature on diseases of sorghums, references to rust caused by *Puccinia purpurea* Cke. are very limited and little is known concerning the occurrence of that disease in North America. It, therefore, was interesting to note outbreaks of rust on sorghums at Manhattan, Kansas, and near La Fayette, Indiana, in 1927. At Manhattan natural epiphytotics of rust developed in varietal sowings in the field in 1927, 1928, and 1929, while infections were artificially induced in varietal sowings at La Fayette in 1928 and 1929. Therefore, it was possible to make comparative rust readings on varieties at both stations and to note differences in susceptibility. The data secured at La Fayette in 1927 already have been briefly presented by Mains (5). The detailed results of experiments at both stations are presented in this paper.

DISTRIBUTION OF THE DISEASE

Sorghum rust usually has been considered a disease of southern distribution and seldom has been reported as far north as Kansas and Indiana. It has frequently been reported from Louisiana and Texas and occasionally from Oklahoma, but usually only from the more humid sections of those States. It is of interest to note that rust is seldom seen in the heavy sorghum-producing sections of northwestern Texas and Oklahoma and southwestern Kansas. Although the temperature undoubtedly is favorable for the rust in that area, it is much too dry for its satisfactory development over most of the area in a normal season. The senior writer noted a small amount of rust on sorghum varieties at the Woodward, Oklahoma, field station in 1927, but that was an unusually wet season in northwestern Oklahoma.

The rust is rather widely distributed throughout the warm temperate and tropical portions of the world. The Sydows (7, pp. 803–805) list *Puc-*

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cinia purpurea from Italy, Greece, Portugal, India, Java, and North America. Arthur (1) also lists it from South America. In North America, Arthur and Fromme (3, pp. 284–285) list it from Alabama, California, Florida, Louisiana, South Carolina, Texas, Guerrero, Vera Cruz, Yucatan; Cuba; Jamaica; Costa Rico; Bermuda; Porto Rico.

CHARACTERISTICS OF THE FUNGUS

Puccinia purpurea probably is heteroecious but its aecial host is unknown. Uredinia are produced, usually in purplish spots, on both sides of the leaf. They are rather small and somewhat tardily naked. The urediniospores are cinnamon or dark chestnut brown, 23–31 by 29–40 μ, finely and rather closely echinulate, with 5–10 germ pores that either are scattered or variously arranged in two transverse bands. The uredinia contain conspicuous clavate or capitate paraphyses, figure 1, which usually

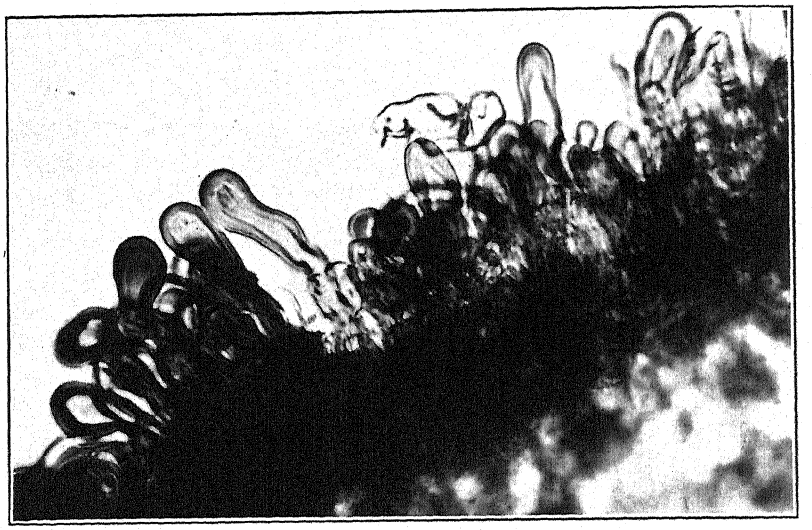


FIG. 1. Section through uredinium from feterita showing paraphyses characteristic of *Puccinia purpurea*.

have a brownish purple wall and are generally more numerous at the margin of the uredinium. The compact and dark chocolate brown telia are somewhat larger than the uredinia. The teliospores are ellipsoid or oblong, 23–32 by 40–50 μ.

Arthur and Fromme (3) list *Holcus halapensis* L. (Johnson grass), *H. sorghum* L. (sorgo) and *H. sorghum sudanensis* (Piper) Hitchc. (Sudan grass) as hosts for this rust. According to the Sydows (7), Barclay described a rust from *Pennisetum typhoideum*, which, from Barclay's de-

scription, is identical with *Puccinia purpurea*. Butler (4, pp. 206–208), however, questions the identity of the host and rust. The Sydows (7) also list *Zea mays* as a host from Natal, Africa. This also would appear somewhat questionable since *P. purpurea* has never been reported on maize elsewhere. The rust of maize, *P. sorghi* Schw., may produce intense purplish spots under certain conditions on maize carrying anthocyanine factors. *Puccinia purpurea,* however, is easily distinguished from *P. sorghi* by its characteristic paraphyses.

In this connection it is interesting to note that the rust of maize was described by Schweinitz (6, pp. 141–316) in 1834 from material that he apparently assumed was sorghum (2). He consequently gave it the name *Puccinia sorghi*. The rust of maize never has been found on sorghum. Although the name was applied evidently through a mistake in identification of the host, a strict adherence to the rules of nomenclature has resulted in a perpetuation of this misnomer and necessitates the application of another name to the rust of the sorghums.

SORGHUM RUST IN THE SOUTHERN GREAT PLAINS

The first occurrence of rust on sorghums in Kansas was recorded at Manhattan on August 20, 1927, when a rather heavy infection was noted on the lower leaves of sorghum varieties in experimental sowings. A more severe outbreak occurred in the same plots in 1928 and this was followed by a much lighter infection in 1929. Weather conditions favored the development of sorghum rust in the southern great plains in 1927 and 1928, both seasons being marked by heavy rains in late summer.

The disease developed very late in each of the three seasons at Manhattan; the dates of first appearance being August 20, 1927, August 13, 1928, and August 26, 1929. The first signs of infection appeared as scattered uredinia on leaves near the ground. During the remainder of the season the rust developed slowly but with such severity that the lower leaves of susceptible varieties were completely killed and the upper leaves also were severely affected. In all cases rust continued to develop until the host plants were killed by frost, the rate of development depending upon environmental conditions and the susceptibility of the sorghum variety.

The amount of injury caused by rust on sorghums is problematical. At Manhattan even those varieties most severely rusted yielded an abundance of seed of good quality in each of the three years. The belated development of rust and the ability of most sorghums to continue producing new leaf tissues through tillering and branching practically preclude the possibility of losses such as those resulting from rust in small grains. On the other hand, when most of the leaves are prematurely killed by rust,

as occurred in certain feteritas, Red Amber × feterita hybrids, and Manchu Brown kaoliang in 1927 and 1928, there can be no doubt that considerable reduction in yield results.

Rust readings were made on a total of 121 sorghum selections grown at Manhattan, Kansas, during 1927, 1928, and 1929. These consisted of inbred lines of varieties, hybrids, and selections then being used in studies on the physiologic forms of covered kernel smut. Not all of the 121 selections, however, were grown each of the three years; 42 of them had been grown only one year and 17, two years. The remaining 62 selections were grown in all three years. In most cases the selections had been inbred for several years, but a few varieties had been inbred only a year or two. Particular care was exercised in the inbreeding operations to select plants having characteristics typical of the named variety. During the growing seasons any obviously off-type plants were promptly removed from the plots. Thus a set of selections of very uniform type was available for study. In some of the hybrid lines it was very difficult to select strains that would react the same from year to year. This apparently was because of the fact that they were still heterozygous for certain characters, although homozygous for gross morphologic characters. In general, however, the rust readings indicated that the selections were uniform for their reaction to rust.

The varieties were grown in 50-foot rows with plants spaced about 4 inches apart. Many plants of each variety therefore were available for examination. Since the varieties were sown each year in triplicate it was possible to make several readings on each variety. In 1927 all three series were examined and a single note recorded, giving the maximum and minimum percentage noted for the variety. In 1928 and 1929 notes were taken on each variety in each of the three series. From these data the range (minimum to maximum percentage) for each variety was calculated. Thus, the data presented in table 1 give the extremes noted for each variety each year.

The rust percentages noted for some of the varieties are very consistent for all of the years, while in other cases the results differed widely from year to year. It must be remembered that 1929 was a rather unfavorable year for the development of sorghum rust and that only the most susceptible selections had high rust readings. Moderately susceptible varieties had very low readings, frequently only a trace of rust being noted on them. The readings for 1927 were taken a little too early and therefore probably are a little lower than they should be. The readings of 1928 are undoubtedly the most accurate and therefore the best measure of resistance or susceptibility of a variety.

In presenting the data in table 1 the varieties, hybrids, and selections have been arranged into large groups such as kafir, sorgo, feterita, milo, etc. Thus, all closely related varieties are conveniently placed for comparison. The kafirs, as a rule, are only moderately susceptible to rust, the lower leaves frequently showing 20 to 40 per cent, while the upper leaves seldom show more than a trace. The data seem to indicate that Pink kafir is slightly more susceptible than Blackhull and that Club kafir probably is the most susceptible variety of the group. The latter probably is not a pure kafir, but a hybrid between kafir and feterita.

The sorgos differ considerably in their reaction to rust, some being rather susceptible, while others apparently are resistant. Red Amber, Freed, Kansas Orange, Leoti Red, and Dwarf Freed seem moderately susceptible, while Pink Freed and Honey sorgo are more resistant. The light infection of 1929 makes certain varieties seem less susceptible than they actually are. If all three years are considered, Dwarf Freed and Freed were the most susceptible of the sorgos, while Pink Freed was the most resistant. The difference in reaction of the two strains of Freed, Pink Freed, and Dwarf Freed, is very interesting. The former is a supposed natural cross between Freed and Pink kafir, while the latter is a dwarf selection from Freed made by A. F. Swanson at Hays, Kansas. The proper classification of Pink Freed is uncertain, since it is not a typical sorgo and cannot be placed in any of the other known groups.

The feteritas as a group were very susceptible to rust and, with few exceptions, the data in table 1 show this very clearly. The only evidences of resistance in this group were shown by a recent introduction, S. P. I. No. 51991, in 1927, and one of the strains of Red Leaf feterita, in 1929. The latter is a selection from normal feterita made by J. H. Parker. The entire plant turns a purplish red late in the season and is characteristically lacking in vigor. In 1929 this strain became red before rust infection occurred and very little rust developed thereafter. Another strain of Red Leaf feterita is much more vigorous and, although originally characterized by red leaves, it developed very little of the color in 1928 and 1929. It was scarcely distinguishable from normal feterita and shows the susceptibility characteristic of the variety.

Not only are the feteritas very susceptible to rust, but many of the hybrids having feterita as one parent also are very susceptible. Most of the Red Amber × feterita hybrids tested were very susceptible, although one of them seemed highly resistant in 1929. These selections were then in the F_3 generation. They are tall, juicy-stalk, white-seed segregates, combinations of forage and grain type. The kafir × feterita hybrids and milo × feterita hybrids also were moderately susceptible, although one selection of the latter had very little rust in 1929.

TABLE 1.—*Reaction of varieties of sorghum to Puccinia purpurea in three seasons at Manhattan, Kansas*

Variety	Serial[a] number	Range in rust percentage		
		1927	1928	1929
Kafir				
Reed	KB 2502	tr.–5	tr.–20	tr.–tr.+
Pink	KB 2506	10	tr.–40	tr.–5
"	KB 2546	40	tr.–40	10
Juicy Pink	FCI 9091	.....	tr.–20	10
Early Pink	KB 2824	.....	tr.–40	tr.+
Wonder	KB 2520	40	.....	.....
"	KB 2548	20	.....	.....
Sunrise	KB 2523	5–30	5–60	tr.
Blackhull	KB 2535	tr.–10	tr.–40	tr.
"	KB 2539	40	.....	.....
Dawn	KB 2538	tr.–10	tr.–20	tr.
"	HC 2421	20	tr.–40	5
Red	KB 2545	tr.–5	tr.–40	5–10
Farmer	KB 2554	30	.....	.....
Western Blackhull	HC 1462	5–20	tr.–40	tr.+
Bishop	KB 27103	tr.–2	.....	.....
Pearl	KB 27104	tr.–10	.....	.....
Rice	KB 27105	20	.....	.....
Sharon	KB 27106	40	.....	.....
Club	HC 281	.....	tr.–40	10–25
Sorgo				
Red Amber	KB 2504	30	20–60	tr.+
Freed	KB 2519	tr.	40–80	10
White African	KB 2521	30	.....	.....
Dwarf Sumac	KB 2576	40	10–40	tr.–
Sumac	KB 2902	.....	.....	tr.–
Kansas Orange	KB 2560	40	20–60	tr.–
"	KB 2572	40	.....	.....
Lasley	KB 2571	5	.....	.....
Leoti Red	FCI 6610	40	20–60	0–tr.–
Pink Freed	KB 2798	tr.–2	0–5	0–tr.–
Modoc Pink Freed	HC 2520	.....	tr.–40	10
Honey	KB 2874	30	tr.–20	0–tr.–
Dwarf Freed	KB 27107	30	40–60	20–40
"Japanese Honey Drip"	KB 2876	.....	tr.–40	5
Atlas Sel. 95	KB 2877	.....	tr.–40	5
" 100	KB 2878	.....	tr.–40	5
Feterita				
Spur	KB 2540	tr.–10	50–80	40
Red Leaf	KB 2543	tr.–20	50–80	tr.–5
"	KB 2544	tr.–20	60–80	25–40
Standard Feterita	CI 182–1	50	40–70	40
"	KB 2563	25	60–80	40–60
Standard Feterita Sel.	SPI 51989	30	50–80	25–40

TABLE 1.—*(Continued)*

Variety	Serial number	Range in rust percentage		
		1927	1928	1929
" "	SPI 51989	5–60	.....	.....
" "	SPI 51991	tr.–2	.....	.....
" "	SPI 51991	40	.....	.....
Hybrid Dwarf No. 6	KB 2820	.....	60–90	40–60
Milo				
Dwarf Yellow	KB 2511	5	tr.–5	0–tr.
"	KB 2512	tr.–2	.....	.....
"	KB 2514	tr.–10	.....	.....
"	KB 2515	tr.–5	0–tr.	.....
"	KB 2516	tr.–2	.....	.....
"	KB 2517	tr.–2	.....	.....
"	KB 2532	tr.	.....	.....
"	KB 2534	tr.–2	.....	.....
"	CI 332	tr.–10	0–tr.	0–tr.–
"	KB 2556	tr.–20	.....	.....
"	KB 2557	tr.–20	.....	.....
"	KB 2564	tr.–20	.....	.....
Standard Yellow	CI 234	5	0–tr.	0–tr.–
" White	CI 352	tr.–5	0–tr.	0–tr.–
Dwarf White	FCI 8927	5	0–tr.	0–tr.–
Cream	KB 2569	5	0–tr.	0–tr.–
Dwarf Straightneck[b]	KB 2844	.....	0–tr.	tr.–
Erect milo[c]	KB 2845	.....	tr.–5	tr.–tr.+
Fargo Straightneck	CI 809	10	0–tr.	0–tr.–
Kaoliang				
Dwarf Shantung	CI 293	40	0–tr.	0–tr.–
Manchu Brown	CI 191	60	50–80	40–65
Broomcorn				
Evergreen	CI 583	0–tr.	tr.–20	tr.
Acme	CI 243	20	tr.–40	tr.
Scarborough	CI 817	.....	20–60	20–60
Black Spanish	CI 826	.....	.....	20–60
Miscellaneous varieties and hybrids				
Weskan	KB 2522	tr.–2	tr.–20	5
Dwarf Hegari	KB 2518	20	5–60	20
"	KB 2537	tr.–5	20–60	20
White Yolo	KB 2525	40	tr.–40	0–tr.–
" durra	CI 81	tr.–5	40–70	20
Darso	KB 2536	tr.–2	tr.–40	10–20
Schrock	KB 2541	tr.–2	tr.–40	0–tr.–
Shallu	CI 85	tr.–2	0–tr.	0–tr.–
"	KB 2879	.....	0–tr.	0–tr.–
Pierce kaferita	KB 2547	tr.–20	20–40	tr.–10
"	KB 2549	tr.–5	tr.–30	tr.+
"	KB 27100	30	.....	.....
"	KB 27101	20	tr.–40	.....

TABLE 1.—*(Continued)*

Variety	Serial number	Range in rust percentage		
		1927	1928	1929
Premo (feterita × kafir)	FCI 8929	30	tr.–20	tr.–5
Chiltex (" ")	FCI 8917	.....	0–tr.	.....
Red Amber × feterita	KB 2501	25	60–80	20–30
" "	KB 2503	60	.....	.....
" "	KB 2507	60	.....	.....
" "	KB 2508	50	.....	.....
" "	KB 2509	25	20–60	tr.–
" "	KB 2513	25	30–70	15–25
" "	KB 2526	60	.....	.....
" "	KB 2527	40	.....	.....
" "	KB 2529	5–60	.....	.....
" "	KB 2530	30	.....	.....
" "	KB 2552	20	10–60	20–25
" "	KB 2562	60	40–70	25–40
" "	KB 2567	50	20–60	25–40
" "	KB 2570	40	40–70	5–40
" "	KB 2573	60	20–60	20–25
" "	KB 2574	40	.....	.....
Blackhull × Sourless	KB 2505	40	tr.–40	.....
" "	KB 2799	5–20	.....	.....
Kafir × feterita	FCI 8920	40	20–60	20–40
" "	FCI 8929	35	.....	.....
" "	HC 2423	40	tr.–40	.....
Milo × feterita	FCI 8926	25	20–40	tr.–
Kafir × milo 26–3–1–1	KB 2561	20	tr.–20	tr.–
" " 38–1–2–1	KB 2679	40	5–40	tr.–5
Smith's milo × kafir	CI 808	tr.–10	.....	.....
Kansas Orange × Dwarf Yellow milo	KB 2680	tr.–2	0–5	0–tr.
" "	KB 2681	10	tr.–40	0–tr.
" "	KB 2682	40	.....	.....
" "	KB 2683	40	.....	.....
" "	KB 2684	tr.–5	0–5	0–tr.
" "	KB 2685	tr.–5	.....	.....
Dwarf Yellow milo × Pink kafir	HC 244	tr.–5	tr.	tr.–
" "	HC 257	tr.–5	tr.–40	tr.–
" "	HC 2510	20	tr.–40	tr.–
Feterita hybrid (milo × feterita)	FCI 8926	30	40–80	.....
Sudan grass		.....	.....	tr.–

[a] KB, HC, FCI, CI, SPI indicate accession numbers of the Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station; Fort Hays Branch Experiment Station, Hays, Kansas; and of the offices of Forage Crops and Diseases, Cereal Crops and Diseases, and Foreign Plant Introduction, of the United States Department of Agriculture, respectively.

[b] A milo type segregate from a kafir × milo 332–1 cross received from J. B. Sieglinger, Woodward, Oklahoma.

[c] A straightneck milo type resembling Fargo Straightneck but much later maturing. Seed from E. E. Senser, Bison, Kansas, who stated he obtained seed in Oklahoma.

As a group, the milos are highly resistant to rust. The highest percentages were noted on varieties of milo in 1927, but in no case was more than 20 per cent seen. A few lowermost leaves of the plants had a moderate amount of rust in that season, but most leaves showed only a very small amount of rust. In many cases but few scattered uredinia were present. In 1928 and 1929 most varieties of milo showed only an occasional uredinium, although one selection of Dwarf Yellow milo and one of Erect milo had an infection of tr-5 per cent in 1928. Hybrids having milo as one of the parent varieties varied considerably in their reaction, although most of them were somewhat resistant. Milo × feterita hybrids seem more susceptible than milo × kafir, Kansas Orange × Dwarf Yellow milo, and Dwarf Yellow milo × Pink kafir hybrids. The two latter crosses contain several highly resistant selections.

Only two varieties of kaoliang were tested at Manhattan. Dwarf Shantung was very highly resistant in 1928 and 1929, while Manchu Brown was highly susceptible all three seasons. In 1927 Dwarf Shantung had considerable rust, especially on certain plants. It is possible that a resistant plant was selfed in 1927, thus establishing a resistant pure line. This is not known to be the case, however, and it may be that a difference in physiologic forms is responsible for the difference in reaction in different years. It is, however, certain that Manchu Brown is a very susceptible variety. It is also extremely susceptible to bacterial stripe. In each of the three years the leaves of this variety were prematurely killed by a combination of extremely heavy infections of rust and bacterial stripe and were thoroughly dry more than half way up the stalk.

Varieties of broomcorn seem to differ considerably in their reaction to rust. Evergreen (standard), CI No. 583, and Acme (dwarf), CI No. 243, were resistant or moderately resistant, while Scarborough and Black Spanish were susceptible. Evergreen, however, seemed to be more resistant than Acme. Thus we have one standard type (Evergreen, C. I. No. 583) resistant and another standard type (Black Spanish) susceptible. The same condition obtains in a general way for the dwarf types. Acme is moderately resistant, while Scarborough is susceptible.

Among the miscellaneous varieties Dwarf hegari, White durra, and darso seemed to be rather susceptible. In 1928 White durra was very susceptible and, in general reaction, the variety seemed to be the same in 1929, although the rust percentage was not high. White Yolo was moderately susceptible in 1927 and 1928 but did not show much infection in 1929. The susceptible reaction of this variety is interesting, since, in several other ways, such as resistance to covered kernel smut, it is very similar to milo. The two selections of shallu were highly resistant to rust in all of the seasons in which they were studied. Shallu seems to be the

most resistant sorghum encountered at Manhattan in the course of these studies. It does not develop red or purplish anthocyanin like most of the sorghums and rust uredinia are therefore not accompanied by the usual red necrotic areas. Several other varieties show the same character to a lesser extent. Leoti Red rarely develops any anthocyanin and there is seldom any in Freed sorgo. The milos, in the field, also show reddening less frequently than most other groups and bear uredinia unaccompanied by red, necrotic areas.

The results of the three years' observations on sorghums at Manhattan very clearly show wide varietal differences in reaction to rust. General statements concerning the reaction of varieties comprising the various groups can be made only with reservations. It can be stated with only occasional exceptions that the kafirs and sorgos are moderately susceptible, the feteritas are very susceptible, and the milos highly resistant. The kaoliang, broomcorn, and miscellaneous groups contain both resistant and susceptible varieties.

The occurrence of *Puccinia purpurea* on sorghum has been reported frequently from Oklahoma, but no data can be found on the reaction of varieties to rust in that State. One of the writers visited the agricultural experiment stations at Stillwater and Woodward, Oklahoma, in the fall of 1927, and there he made a few observations on sorghum rust.

At Stillwater, on September 18, most of the varieties of sorghum had been harvested and only a few were available for study. Rust infection seemed to have been rather light there that season and only small percentages were noted on Blackhull kafir, darso, and feterita.

The amount of rust infection noted at Woodward, on September 19, seemed somewhat higher than that at Stillwater. This was interesting since Woodward is a typical dry-land station, where the average annual rainfall is about 24 inches. The rainfall in the late summer of 1927, however, seems to have been sufficient for the development of considerable rust on sorghum. The amount of infection even on the most susceptible varieties was by no means so heavy as that noted at Manhattan, and it is doubtful if the notes collected do more than point out the susceptible strains. Rust notes were taken on 39 varieties and strains. Blackhull kaoliang and African kafir, CI No. 802, seemed to be very susceptible, while Dwarf feterita, Pink kafir, Sunrise kafir, darso, and Shantung kaoliang were only moderately susceptible. Early Red, Wonder, and Bishop kafirs, and Sourless (African Millet) and Sumac sorgos were free from rust. The amount of infection, however, was too low to render a zero reading very significant. In general, the observations made at Woodward agree with those made at Manhattan, but the percentages at the former station were too low to permit more than a very general comparison.

SORGHUM RUST IN INDIANA

The only observation of sorghum rust in Indiana was made in 1927, when Dr. M. W. Gardner collected this rust in one spot in a field in Tippecanoe County, on October 19. Infection in this limited area was severe. The field was planted with Sagrain (Schrock) sorghum from seed obtained from Texas.

Since this rust had received very little investigation, it was decided to make a study of the relative susceptibility of sorghum varieties received from the Kansas Agricultural Experiment Station and the Office of Cereal Crops and Diseases. This study was made in the field during the summers of 1928 and 1929 and upon seedlings in the greenhouse during the winters of 1927–1928, 1928–1929 and 1929–1930. The results are given in tables 2 and 3.

In 1929 a number of additional varieties were received from the Kansas Agricultural College, which were studied only in the seedling stage in the greenhouse. These reacted as follows:

Very susceptible, the uredinia accompanied by pronounced purpling; Acme broomcorn CI 243, Scarborough broomcorn KB 30107, Blackhull kafir KB 2539, Club kafir KB 2828, Early Pink kafir KB 2824, Farmer kafir KB 2554, Pink kafir KB 2506, Pink kafir KB 2546, Reed kafir KB 2502, Sunrise kafir KB 2523, Western Blackhull kafir KB 27102, Feterita KB 2690, Red Leaf feterita KB 2544, Atlas sorgo KB 2877, Kansas Orange sorgo KB 2572, Kansas Orange sorgo KB 2560, White African sorgo KB 2521, Manchu Brown kaoliang CI 171, Pierce kaferita KB 2547, Premo FCI 8929, Sharon kafir KB 27106, White Yolo KB 2525, Kafir × milo 38–1–2–1, Kansas Orange × Dwarf Yellow milo KB 2681, Red Amber × feterita KB Nos. 2501, 2509, 2513, 2552, 2562, and 2570.

Very susceptible, the uredinia accompanied by moderate purpling; Evergreen broomcorn CI 583, Juicy Pink kafir FCI 9091, Red kafir KB 2545, Pink Freed KB 2798, Dwarf Yellow milo × pink kafir HC 257.

Moderately susceptible the uredinia accompanied by pronounced purpling; Blackhull kafir KB 2569, Dawn kafir KB 2538, Dawn kafir HC 2421, Dwarf Sumac sorgo KB 2576, Honey sorgo KB 2874, Weskan sorgo KB 2522, Wonder kafir KB 2520, Wonder kafir KB 2548, darso KB 2536, Pearl kafir KB 27104, Pierce kaferita KB 2549, Pierce kaferita KB 27101, milo × feterita FCI 8926, kafir × feterita HC 2423, Red Amber × feterita KB 2567 and KB 2573.

Moderately susceptible, uredinia accompaniel by moderate purpling; Dwarf Freed sorgo KB 27107, Freed sorgo KB 2519, Modoc Pink Freed sorgo HC 2520, Red Amber sorgo KB 2504, Dwarf hegari KB 2518, Dwarf hegari KB 2537, Rice kafir KB 27105.

Moderately susceptible, uredinia not accompanied by purpling; "Japanese Honey Drip" sorgo KB 2896, Leoti Red sorgo FCI 6610, shallu CI 85, White durra CI 81.

Very resistant, uredinia few, small, accompanied by pronounced purpling; Bishop kafir CI 814, milo × feterita FCI 8926.

Highly resistant, no uredinia produced, infection evident as small purplish flecks; Dwarf White milo FCI 8927, Dwarf Yellow milo KB 2515, Dwarf Yellow milo KB 2556. Dwarf Yellow milo KB 2564, Fargo Straightneck milo CI 809, Standard White milo CI 352, Standard Yellow milo CI 234, Lasely sorgo KB 2571, Dwarf Shantung kaoliang CI 293, Schrock KB 2541, Dwarf Yellow milo × Pink kafir HC 244, HC 2510, Kafir × milo 26–3–1–1, Kansas Orange × Dwarf Yellow milo KB 2680, Kansas Orange × Dwarf Yellow milo KB 2684, Smith's milo × kafir CI 808. Shallu KB 2879 was highly resistant, showing only a few very indistinct flecks.

In both field and greenhouse tests at Purdue University all the strains of Milo (Fig. 2, A, and Fig. 3) were highly resistant to *Puccinia purpurea*.

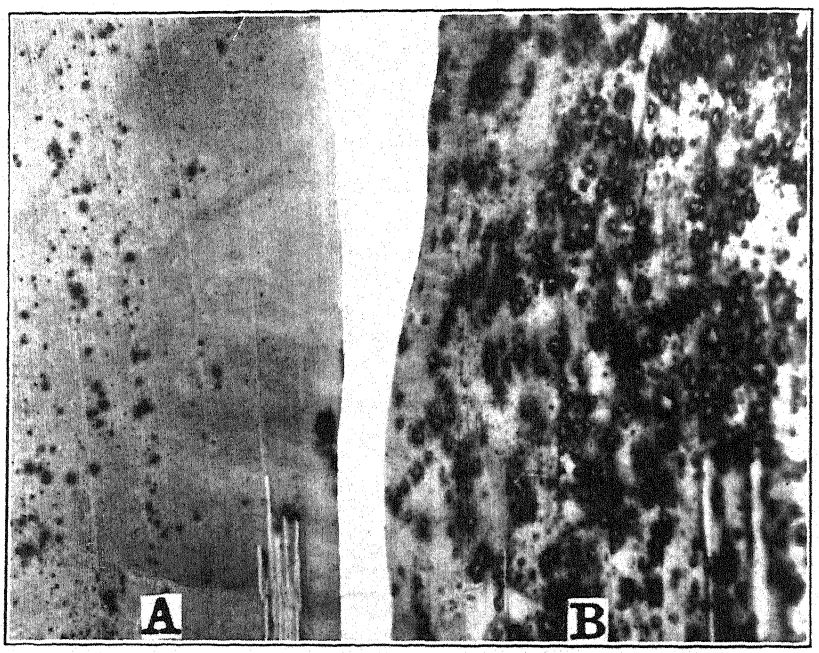


FIG. 2. A. A portion of a leaf of the highly resistant Dwarf Yellow milo showing small purplish flecks without uredinia. B. A portion of a leaf of very susceptible feterita showing large uredinia surrounded by large purplish spots. Leaves from field plantings, La Fayette, Indiana, 1928.

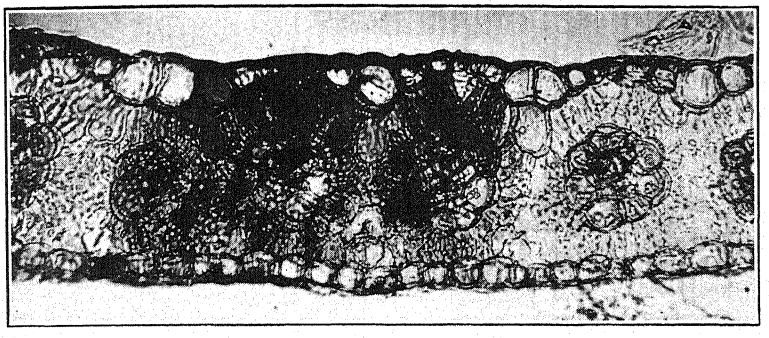


FIG. 3. Section through leaf of very resistant milo infected with *Puccinia purpurea* showing purplish discoloration of the small infected area and absence of uredinia. Compare with figures 6 and 7.

Dwarf Shantung kaoliang KB 2565 (CI 293) (Fig. 4, B), Lasley sorgo KB 2571, Bishop kafir CI 814, shallu KB 2879 (Fig. 4, C), Schrock KB 2541 and hybrid selections HC 244, HC 2510, KB 2561, KB 2679, KB 2684, KB 2678 from crosses with milo were the most outstanding for resistance in

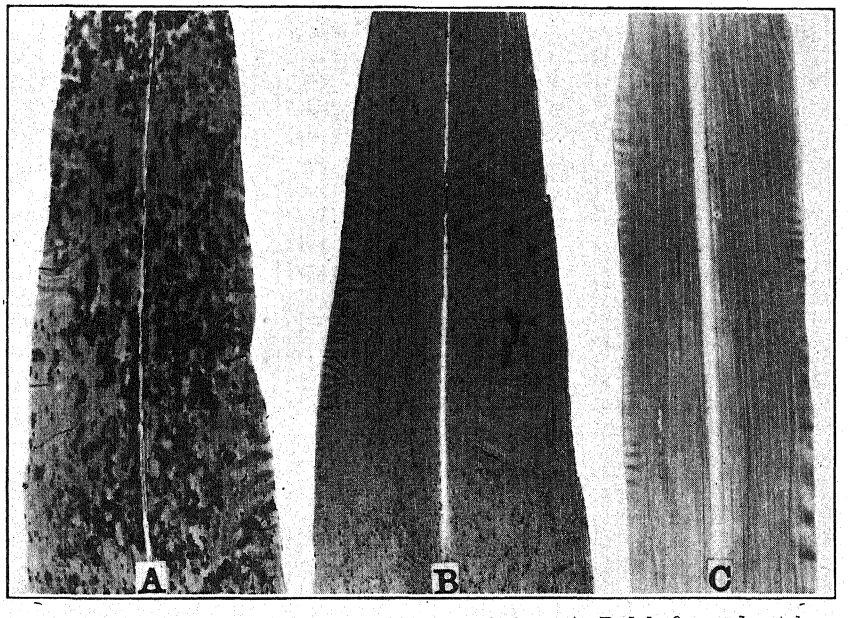


FIG. 4. Comparative reaction of sorghum varieties. A. Red kafir, moderately susceptible with large uredinia in purplish spots. B. Dwarf Shantung kaoliang, very resistant, infection indicated only by slight purplish flecks, no uredinia. C. Shallu, very resistant, infection indicated only by extremely few indistinct flecks, no uredinia. Leaves from field plantings, La Fayette, Indiana, 1928.

TABLE 2.—*Relative reaction of sorghum varieties to Puccinia purpurea in field and greenhouse tests at La Fayette, Indiana. 1928–1929*

Variety	Acc. No.[a]	Rust reaction		
		Field[b]	Greenhouse[c]	
		1928	1928	1929
Kafir				
Reed	KB 2502	tr.–25P	4P	3P
Sunrise	KB 2523	50–100P	4P	3P
Blackhull	KB 2535	5–15P	4P	
Dawn	KB 2538	25–50P	4P	3p
Red	KB 2545	75–100P	4p	
Reed	CI 628	15–35P	4P	3p
Red	CI 34	35–65P	3–4P	4p
Dawn	CI 340	50–65P	3–4P	4P
Blackhull	CI 310	50–100P	4P	3P
Sorgo				
Freed	KB 2519	50–65P	3–4p	3p
White African	KB 2521	50–65P	3–4p	
Fielding Sumac	KB 2553	50–100P	4P	3P
Kansas Orange	KB 2560	15–25P	3–4P	3P
Leoti Red	KB 2575	50–100	4	3
Dwarf Sumac	KB 2576	75–100P	3–4P	
Red Amber	FCI 17548	50–75P	4p	4P
Leoti Red	FCI 6610	50–65P	3–4	3
Kansas Orange	FCI 9108	35–50P	4P	4P
Freed	CI 350	50–65P	3–4P	
Saccaline	FCI 48191	25–50P	3–4P	4P
Sourless	FCI 9074	50–75P	4P	4P
Atlas	FCI 9112	50–65P	4P	4P
Black Amber	FCI 7038	50–100P	4P	4P
Feterita				
Standard feterita	KB 2563	75–100P	3–4P	
"	SPI 51989	50–75P	4P	4P
"	SPI 51991	50–75P	4P	3P
"	CI 182–1	75–100P	4P	3P
"	CI 182	50–100P	4P	3P
Milo				
Dwarf Yellow	KB 2512	0P	0P	tr.P
Standard Yellow	CI 234	0–tr.P	0P	0P
" White	CI 352	0p	0P	0p
Fargo Straightneck	CI 809	tr.–15P	0P	
Dwarf Yellow	CI 332	tr.–15P	0P	0p
Kaoliang				
Dwarf Shantung	CI 293	0P	0P	0p

TABLE 2.—(*Continued*)

Variety	Acc.[a] No.	Rust reaction		
		Field[b]	Greenhouse[c]	
		1928	1928	1929
Broomcorn				
Acme	KB 2558	15–25P	3–4p	3P
"	CI 243	50–75P	4p	4P
Miscellaneous				
Shallu	CI 85	0	3–4	
Dwarf Hegari	KB 2518	50–65P	4P	3P
"	CI 620	50–100P	4P	
Schrock	KB 2541	75–100P	4P	4P
"	CI 616	25–35P	3–4P	3P

[a] Accession numbers of the Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station, and of the offices of Forage Crops and Diseases, Cereal Crops and Diseases, and Foreign Plant Introduction, of the United States Department of Agriculture.

[b] Percentage of rust, the total amount possible being taken as 100%. Notes taken in September.

P = Pronounced purpling accompanying uredinia.

p = Purpling less pronounced. Where no letter is given no purpling was noted.

[c] Numerals indicate type of reaction; 4, very susceptible; 3, moderately susceptible; 2, moderately resistant; 1, very resistant; 0, highly resistant, no uredinia produced.

the other groups studied. Usually no uredinia were produced. Only small purplish flecks indicated that infection had occurred, and sometimes these were very indistinct, as in the case of shallu (Fig. 4, C). Most of the other varieties were more or less susceptible, differing mostly in the number of uredinia produced. This was most marked in the field planting in 1928 (Table 2). Thus, while such varieties as Schrock, Leoti Red sorgo, and Dawn kafir did not differ much in the seedling stage in the greenhouse, in the field they showed considerable difference in amount of rust, as indicated in table 2 and figure 5. A number of the kafirs were only slightly to moderately rusted, most of the sorgos were moderately to heavily rusted, while all of the feteritas were heavily rusted.

As has been noted, infection of the sorghums usually results in the production of a purplish discoloration. This takes place both in resistant and susceptible varieties. Thus, while uredinia are not produced on milo, small purplish spots develop (Fig. 2, A). The purplish areas on susceptible varieties such as feterita are, however, much larger (Fig. 2, B, Fig. 6), probably corresponding to the area occupied by the mycelium. A few

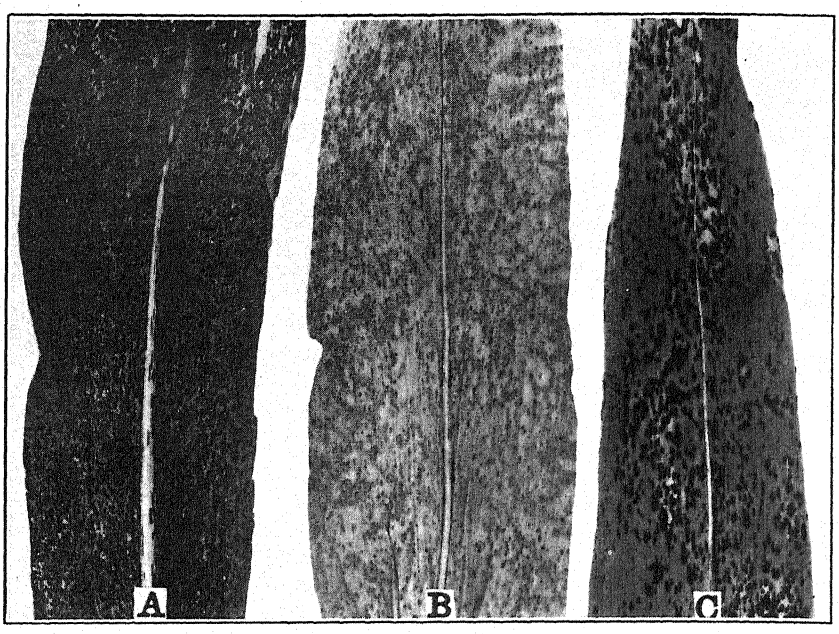


FIG. 5. A. Schrock, very susceptible, uredinia abundant in pronounced purplish spots. B. Leoti Red, moderately susceptible, uredinia fairly abundant, no purpling. C. Dawn Kafir moderately susceptible, uredinia fairly abundant in pronounced purplish spots. Leaves from field planting, La Fayette, Indiana, 1928.

varieties, however, do not develop purplish color in the infected areas. Leoti Red sorgo FCI 6610 (Fig. 5, B, Fig. 7), "Japanese Honey Drip" sorgo KB 2876, shallu CI 85, and White durra CI 81 were more or less susceptible varieties which produced little or no purpling. Shallu CI 85 and KB 2879 (Fig. 4, C), among resistant varieties, showed little or no development of purple in infected areas. The occurrence of purple, therefore, seems not to be due to the effect of the fungus on the host but to the presence of factors for color development in certain varieties.

A few grass sorghums also were studied in 1929 in the greenhouse. Sudan grass KB 2981, FCI 17540, and Johnson grass FCI 15879 were very susceptible, accompanied by pronounced purpling, and Tunis grass FCI 38108 was moderately susceptible, with pronounced purpling. Fifteen unnamed grass sorghums were also received from Mr. H. N. Vinall of the Office of Forage Crops and Diseases, United States Department of Agriculture. Of these, Nos. 50790, 52000, 52022, 52044, 52062, and 52124 were very susceptible, with pronounced purpling; Nos. 49697, 50008, 50079, 52026, 52132, and 58742 were moderately susceptible, with pronounced purpling; Nos. 51995, 52050, and 52053 were highly resistant, no uredinia being formed, infection being evident as slightly purplish flecks.

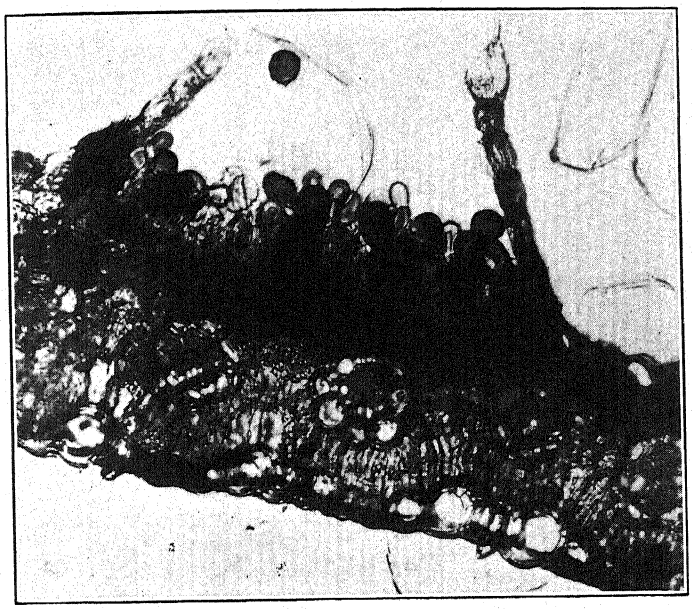


FIG. 6. Section through uredinium of *Puccinia purpurea*, from susceptible feterita, showing paraphyses, urediniospores, and marked purpling of the infected tissue. Compare with figures 3 and 7.

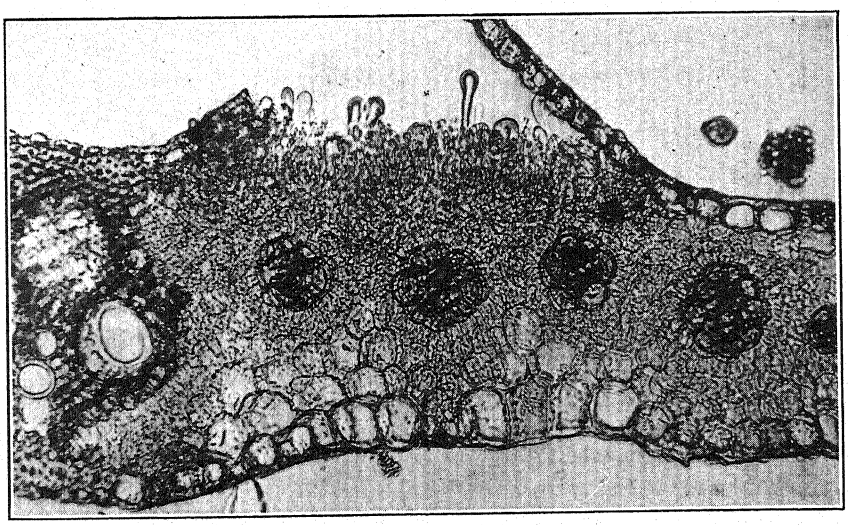


FIG. 7. Section through uredinium of *Puccinia purpurea* from the susceptible variety Leoti Red sorgo showing paraphyses and absence of purplish coloration in the infected tissue. Compare with figures 3 and 6.

TABLE 3.—*Reaction of seedlings of a selected set of sorghum varieties to four cultures of Puccinia purpurea in greenhouse, La Fayette, Indiana. 1930*

Variety	Acc. No.[a]	Culture numbers[b, c]			
		2	3	4	5
Standard Yellow milo	KB 2524	0P	0p	0P	0P
Acme broomcorn	KB 2558	4p	4p	4p	4p
Blackhull kafir	KB 2539	4P	4p	4P	4p
Dawn kafir	KB 2538	..	3p	.....	4p
Dwarf White milo	KB 2566	0P	0p	0P	0p
Dwarf Yellow milo	KB 2515	0P	0P	.....	0P
"	KB 2564	0P	0P	1P	0P
Fargo Straightneck milo	KB 2677	0P	0P	0P	0p
Standard White milo	KB 2533	0P	0P	1P	0P
Kansas Orange sorgo	KB 2572	4p	4p	4p	3p
Lasley sorgo	KB 2571	0P	0p	0P	0p
Dwarf Shantung kaoliang	KB 2565	0p	0p	0p	0p
"	KB 2565 Sel.	0p	0p	0p	0p
Manchu Brown kaoliang	KB 2568	4P	4P	4P	4P
Bishop kafir	KB 27103	0P	0p	1P	0p
Wonder "	KB 2548	4P	4P	4P	4p
Darso	KB 2536	4P	4P	4P	4P
Dwarf hegari	KB 2537	4p	4P	4p	4P

[a] Accession number of the Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station.

[b] Type of reaction. 4, very susceptible; 3, moderately susceptible; 2, moderately resistant; 1, very resistant; 0, highly resistant, no uredinia.

[c] 2 collected at Manhattan, Kansas, on Standard White milo 352, Aug. 29, 1929.
3 collected at Manhattan, Kansas, on feterita CI 182, Aug. 29, 1929.
4 collected at Manhattan, Kansas, on Scarborough, September 3, 1929.
5 collected at La Fayette, Indiana, September 19, 1929.

Corn having been reported as a host for *Puccinia purpurea*, the sorghum rust was sown on 384 inbred lines of corn. These include lines of Howling Mob, Golden Bantam, Golden Rod, and Narrow Grained Evergreen sweet corns, Ball pop corn and Golden Glow, Early Yellow Dent, Reid's Yellow Dent, and Lancaster County dent corns. No signs of infection were noted on these.

To determine whether sorghum varieties might serve as hosts of corn rust, *Puccinia sorghi*, 115 strains and varieties of sorghum were inoculated with physiologic form 1 of *P. sorghi* in the spring of 1929, in the seedling stage, in the greenhouse. These included all of the strains and varieties studied for reaction to *P. purpurea* in 1929. Uredinia did not develop on any of these. In most cases a very faint purplish flecking was noticed.

SUMMARY

Rust (*Puccinia purpurea*) was found on sorghums as far north as La Fayette, Indiana, and Manhattan, Kansas, in 1927. The disease also was seen on sorghums in Oklahoma in the same year.

Rust readings were made on the varieties grown in the field at Manhattan in the seasons of 1927, 1928, and 1929, and in the field and greenhouse at La Fayette in 1928, 1929, and 1930.

Varieties of sorghum were found to vary considerably in their reaction to rust. In general, the kafirs and sorgos seemed to be moderately susceptible, while the feteritas were very susceptible and the milos very resistant.

Both resistant and susceptible varieties were found in kaoliang and broomcorn.

In the field shallu proved to be highly resistant in all years, while White durra was susceptible.

Hybrids between several varieties gave varying reactions, depending upon the varieties used in crossing.

Several unnamed grass sorghums, Johnson grass, Tunis grass, and Sudan grass proved to be susceptible, while several other unnamed grass sorghums were resistant.

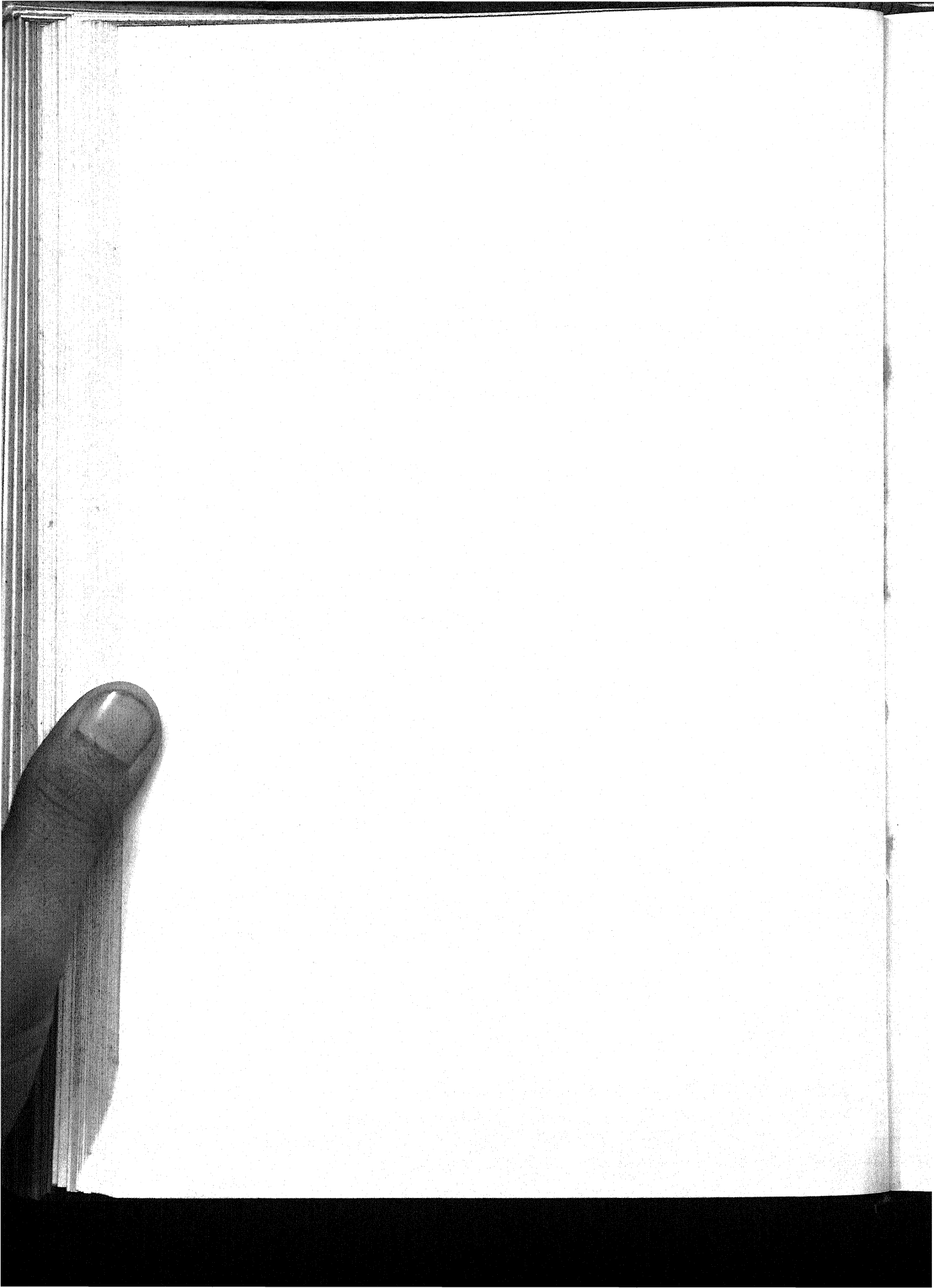
Pronounced purpling accompanied the uredinia on most varieties, but none was observed in shallu CI 85, White durra CI 81, and Leoti Red FCI 6610, and "Japanese Honey Drip" KB 2896 sorgos. These varieties apparently lack factors governing the development of red pigment in foliar tissues.

No definite evidence of the occurrence of physiologic forms was obtained.

Puccinia purpurea failed to cause infection in varieties of dent and sweet corn, and no infection resulted from the inoculation of varieties of sorghum with *P. sorghi* physiologic form 1.

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THE IMPORTANCE OF INVESTIGATIONS ON THE EFFECTS OF KNOWN MIXTURES OF MICROORGANISMS[1]

HOWARD S. FAWCETT

Investigation with one microorganism kept pure and free from contamination with any other has been the classical procedure ever since Koch and others perfected the pure-culture methods that facilitate so greatly the separation of microorganisms. Students in our laboratories have been thoroughly imbued with the idea that cultures must be pure for a single organism. This necessary insistence on pure cultures of single organisms has perhaps led unconsciously to a feeling that to allow the use of a mixture in plant-pathological work is extremely unscientific if it is not actually a "*deadly plant pathological sin.*"

The insistence on purity of cultures was, indeed, a very important condition in preliminary work for the study of the part played by single organisms in the absence of all others. The work with one organism at a time was a necessary stage of analysis. I feel, however, that we have now come to a stage requiring synthesis as well as analysis, integration as well as differentiation. We are not, it would seem, getting the whole plant-pathological story by working solely with pure cultures of single organisms. Nature does not work with pure cultures alone but most frequently with associations. No mathematician would be satisfied to take his student only through differentiation in calculus but would push on to the all-important stage of integration. Rahn (17), in microbiology, pointed out clearly the importance of a study of the mutual influence of microorganisms when he said "The experience obtained with pure cultures is not sufficient to explain all microbial activity in nature."

In view of these considerations I feel we are ready as plant pathologists to enter more actively into the investigation of the effects of known mixtures in comparison with the effect of the individual organisms alone in their relation to disease. Pure cultures we must, of course, continue to use as a basis for the known mixtures and as controls on the activity of the mixtures. Is it not just as scientific and free from criticism for us to work with known mixtures of fungi as it is for a chemist to work with known mixtures of chemical compounds? Numerous examples in chemistry come to mind at once where mixtures in solutions bring about definite things that none of the components can accomplish when acting alone. A classical example is the mixture of acids in *aqua regia* and also the action of numerous catalysts. Research with mixtures of microorganisms will not,

[1] Presidential paper presented at Twenty-second Annual Meeting of the American Phytopathological Society at Cleveland, Ohio, December 30, 1930.

however, furnish an excuse for any less care than with pure cultures in excluding organisms foreign to the given mixtures.

A small beginning in this line of research has been made by various workers, but there appears to be a wide-open field for more investigation in this subject, especially in plant pathology.

There are two phases out of many possible ones in this subject that I believe will be highly productive of results in research: (1) the study in a quantitative as well as qualitative way of the effect of known mixtures or combinations of microorganisms in culture media; (2) the study in the same way of the effect on development of disease by inoculation of plants with known mixtures of microorganisms. A considerable number of publications have appeared bearing directly or indirectly on the first phase of the subject, but only a limited amount of literature has been found on the second.

The literature on the effects of association of organisms in cultures has been recently reviewed by Buchanan and Fulmer (2), especially for bacteria; by Porter (16); and by Machacek (10), especially for fungi. The type of association in which different organisms, growing together, bring about effects which any one of them growing alone is unable to accomplish has been called "synergism" by Holman and Meekison (9). Some striking examples of synergism may be mentioned.

Waksman and Lomanitz (24) have shown that *Bacterium cereus* and *Bact. fluorescens,* growing together, can bring about formation of ammonia from proteins. The first can act only on the protein to form amino acids, the second only on the amino acid to form ammonia.

Sherman and Shaw (21) found that the rate of fermentation of lactose to form propionic acid was speeded up very greatly by the combination of either *Streptoccoccus lacticus* or *Lactobacillus casei* with *Bacterium acidi-propionici.*

Marshall and Ferrand (11) found that more than half of the organisms usually present in milk, when grown in combination with lactic microorganisms, accelerated the growth and the action of these lactic-acid formers.

Cellulose decomposition was found by Sanborn (18) to be greatly aided by the association of certain microorganisms in a cellulose medium with *Cellulomonas folia.* The cells of the associated organisms were thought to furnish some essential food substances which stimulated the growth and physiological efficiency of the cellulose destroyer.

Examples of both accelerated and retarded or inhibited growth in mixed cultures of fungi as compared with growth of one alone were found by Harder (8) and by Zeller and Schmitz (25). Harder has given a good review of the literature preceding 1911. In Alternaria cultures Elliott (4,

p. 463) noted profound effects on the growth and form of the hyphae when certain bacteria were present.

It was determined by McCormick (12) that the fungus *Thielavia basicola,* when grown in pure culture, usually failed to produce perithecia but, when combined with certain other fungus species, perithecia were readily formed. The same relationship between species of Ascobolus and certain bacteria was found by Molliard (14).

Machacek (10), in carrying out a large amount of quantitative work with different pairs of fungi in cultures, found examples of (1) mutual tolerance and (2) partial or complete inhibition of one by its associate, the degree of inhibition depending somewhat on temperature and number of spores. Many other examples of synergism or associated action might be mentioned in connection with bacteria and fungi in cultures.

In the second phase of the subject—that of the effect of known mixtures or combinations of organisms on the occurrence and development of diseases—the literature appears to be rather limited, although the idea is by no means new and, recently, is receiving more attention. Some examples that I have been able to find, including those of my own experiments, will be mentioned.

In Florida, Fawcett (5), found that *Diplodia natalensis* and *Colletotrichum gloeosporioides,* inoculated simultaneously in slight wounds in citrus bark, produced much more marked effect than when each was applied alone. The same was true of a combination of *Phomopsis citri, D. natalensis,* and *Cladosporium herbarum* var. *citricolum.* Later, in California, the same author (6) showed that inoculations of *Phytophthora citrophthora* combined with *Fusarium* sp. produced much more rapidly enlarging lesions of Pythiacystis gummosis than did the inoculation with *Phytophthora citrophthora* alone. The *Fusarium* sp., when introduced alone, was unable to advance at all in this wounded bark. This was a case of a saprophytic organism greatly aiding a parasitic one. The opposite effect, or negative synergism, was shown when mixtures of the walnut Melaxuma fungus, *Dothiorella gregaris,* and the walnut blight bacterium, *Pseudomonas juglandis,* were inoculated into large walnut branches (7, p. 38). The mixture resulted in an almost complete inhibition, no lesion being formed, while *D. gregaris* alone was capable of producing large lesions, some of which girdled and killed the branches.

In line with this latter effect are the results recorded by Millard and Taylor (13) with potato scab. They found that a mixture of a pathogene, *Actinomyces scabies,* and a saprophyte, *A. praecox,* in soil in pots reduced the occurrence of scab sometimes to a negligible amount as compared with action of the parasite when applied alone. Great inhibition in the infection of wheat seedlings was reported by Porter (16) as the result of mixing

certain bacteria in the same soil with Helminthosporium and delay in the infection of flax seedlings by mixing bacteria with Fusarium in the same soil. At the Des Moines meeting of the American Phytopathological Society, Plakidas (15) stated that a mixture of nine strains of Pythium was less severe in producing a root rot of strawberries in Louisiana than any one of the pathogenic strains alone. Bamberg (1) reported at the same meeting that an inoculum containing a mixture of *Ustilago zeae* and a certain bacterium almost entirely failed to produce smut in maize.

Not to neglect the field of virus diseases, a certain type of streak disease of tomatoes has been reported by Dickson (3) and Vanterpool (23) and confirmed by others, to be produced by a mixture of potato-mosaic and tomato-mosaic virus. That other virus mixtures may produce effects not accomplished by either component alone has been recently reported by Valleau and Johnson (22).

The most extended recent work with mixed inoculations appears to be that of Machacek (10) on apple fruit and that of Savastano and Fawcett (19) on citrus fruit. Machacek obtained with apple-rot fungi an increase of decay with some mixtures and a decrease with others, depending to some extent on the temperatures. The results of the mixed inoculations of Savastano and Fawcett (19) showed striking examples of (1) both accelerating and depressing effects of certain mixtures as compared with the effect of any one of the organisms acting alone; (2) the selective effect of temperature, in many cases enabling one organism in a mixture to dominate the others in producing decay; (3) the influence of certain mixtures on changes in color or consistency of decay. Great acceleration was produced by most of the mixtures containing *Oospora citri-aurantii* and some retardation with most mixtures containing *Botrytis cinerea*.

During the past year Savastano and Fawcett (20), in connection with mal secco of citrus in Sicily, found that infection was inhibited or suppressed by a mixture of *Deuterophoma tracheiphila* and *Fusarium* sp., the former alone being able to invade the wood rapidly.

This acceleration or depression of disease caused by a mixture of organisms probably is related in many cases to the combination of enzymes that are present and their action in making food materials for growth available or in producing inhibiting and accelerating substances. The real causes for these phenomena need careful investigation.

I suspect that many plant diseases are influenced by associated organisms to a much more profound degree than we have yet realized, not only as to inhibition, but as to acceleration, of the processes. It may be that a number of diseases may require an association of organisms for their occurrence and cannot be produced by infection of one organism alone.

These considerations appear to indicate an inviting field for much more extended research. Work with mixtures, however, will not make the already complex problem of plant pathology as a whole any easier or less complex, but it may throw much light on certain relationships, relationships which will probably never be discovered by the use of pure cultures of single organisms. We cannot, therefore, in my judgment, avoid entering actively into this largely unexplored field.

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SOIL CULTURES FOR THE LABORATORY PRODUCTION OF SCLEROTIA IN PHYMATOTRICHUM OMNIVORUM[1]

B. F. DANA

The production of true sclerotia in pure cultures of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, was first reported by King and Loomis (2, 3) and later by Neal (4) and Taubenhaus and Ezekiel (6). These workers used pure cultures as sources of inoculum and sterilized mixtures of soil, sand, and plant materials as media. In these cultures, the fungus was not obliged to compete with other organisms. The conditions under which sclerotia developed were entirely artificial and not directly comparable with the conditions under which sclerotia are produced in nature.

It is the purpose of this paper to describe cultures, brief mention of which appeared in an earlier popular report (1), more nearly approximating field conditions than those noted above. The work herein described parallels, in part, that of Taubenhaus and Ezekiel (5, 6).

Non-sterilized field soil of suitable moisture content is used as a medium. In this is placed the inoculum, which consists of newly-diseased roots of one of the many susceptible plants. The use of soil and diseased plant parts without special preparation is especially simple. The results vary with the different materials used and other fungi, as well as insects, are troublesome at times. The ready and abundant production of sclerotia, however, and the adaptability of these cultures to the study of field factors affecting sclerotial production make it seem desirable to describe in detail the methods used and results secured.

MATERIALS AND TECHNIQUE

Containers: Erlenmeyer flasks, damp chambers, and fruit jars of various kinds and many other types of glass jars have been used. Square mason fruit jars (Fig. 1, B) of quart and 2-quart capacity have been used most extensively because of their availability and reasonable cost. The glass container permitted ready examination of the development of the fungus among the exposed soil particles. The square container also permitted better close-up photographs than round or irregular containers and packed more conveniently into incubators and culture chambers. An ordinary

[1] Presented at the 1930 meeting of the Southwestern Division of the American Association for the Advancement of Science and published with the approval of the Director as Contribution No. 126. Technical Series, Texas Agricultural Experiment Station.

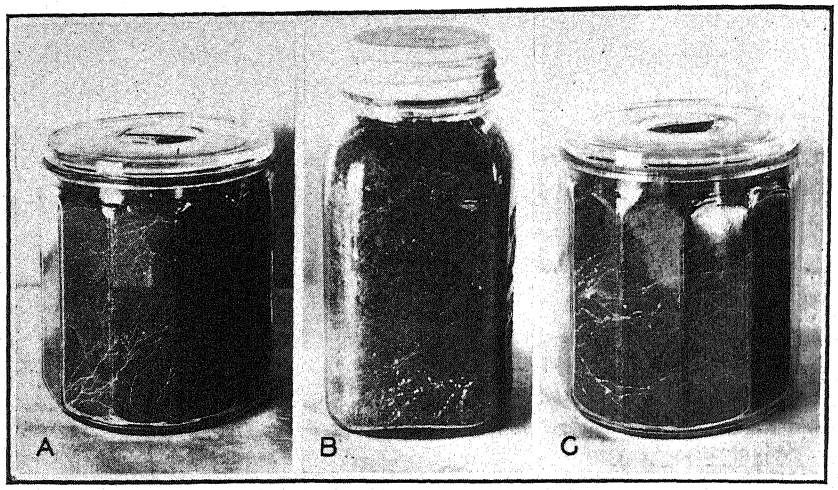


FIG. 1. Soil cultures. A. Cracker jar showing strand growth from cotton inoculum. B. Mason jar showing young sclerotia. C. Cracker jar showing strands and young sclerotia.

commercial cracker jar (Fig. 1, A and C) of about 2-quart capacity provided with loosely fitting cover was used to some extent. In the use of fruit jars the regular mason cover was used without rubbers, for the cultures seemed to develop a little better if the jars were not sealed. A wooden frame with glass sides was constructed for photographic and exhibition purposes. For one experiment a case, much like a museum case, was built up of pieces of glass 18 inches square made tight at the corners with strips of paper. Containers made entirely of wood or metal were not employed because of the difficulty of examining the progress of the culture.

Preparation of cultures: The first cultures to produce an abundance of sclerotia were set up June 5, 1929, in cracker jars of about 1.5 liters capacity and provided with a loose-fitting lid. Houston black-clay soil with a moisture content of about 30 per cent was loosely packed into these jars. Diseased carrots furnished the most readily available infected roots. These were placed in the soil as the jars were being filled. The cultures thus set up were incubated in the laboratory at room temperature, which was then near 27° C.

New strand growth appeared within 24 hours and spread rapidly over the soil particles close to the glass. These strands were, at first, white, later turning darker and finally becoming brownish. At the end of 5 days sclerotia began to appear in these cultures as swellings in the thicker portion of the strands. These newly-formed sclerotia were at first white (Fig. 2, A), turned brownish after a few days, and became reddish brown

(Fig. 2, B) when finally mature. The sclerotia thus formed were variously clustered and lobed and covered with short hyphal branches. In many strands the sclerotia were formed at intervals and presented an appearance not unlike a string of beads. In size these sclerotia ranged from those a little thicker than the strands to those 2 to 3 mm. in diameter. With cotton inoculum many were formed which measured as much as 5 mm. in diameter.

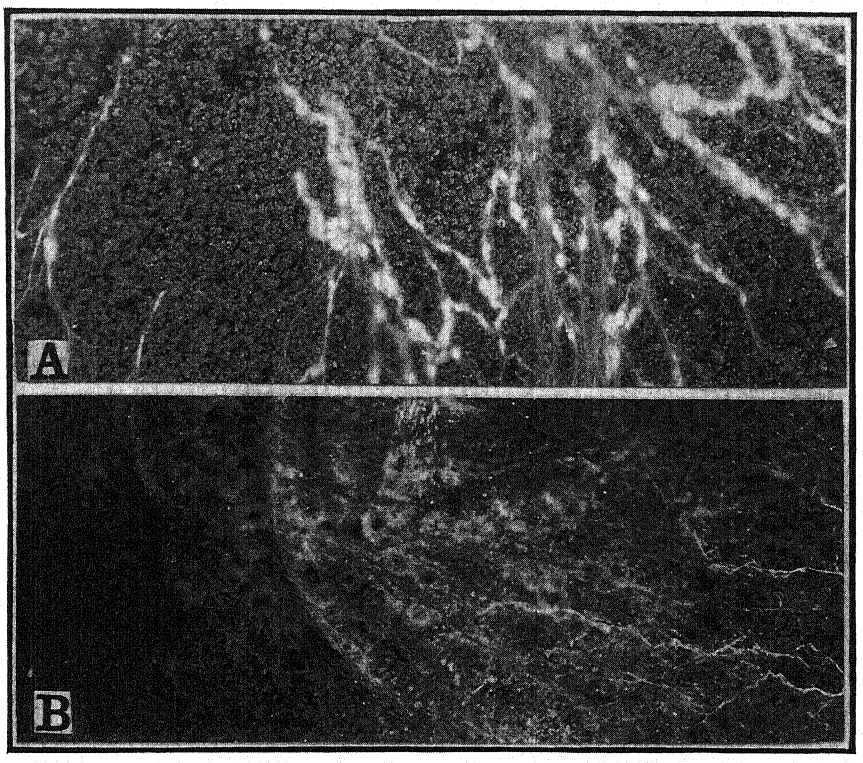


FIG. 2. Sclerotial development in soil cultures. A. Young strands and sclerotia. B. Mature sclerotia.

Inoculum: As already stated, diseased carrots were used as inoculum in the first soil cultures to produce sclerotia. Carrots produced the largest number of sclerotia, while diseased cotton roots produced larger, although fewer, individual sclerotia. Diseased roots of many other plants also were tested, but none of them has compared with cotton and carrot inoculum in the production of large quantities of sclerotia.

The condition of the diseased root was found to be important. Best development of strands and sclerotia was secured from roots with well-

established infections, although with both carrots and cotton the best results were obtained from roots which were not completely covered by the fungus. Roots with incipient infection were found unsuitable, since the fungus failed to make further development after the root was disturbed. Also, roots that had stood in the field until fungus development was checked failed to show any new growth of the organism. This was particularly noticeable in roots procured after the disease was checked by the cooler fall weather. When the fungus was in an active condition it showed a greater tendency to send out strands over the surrounding soil.

Comparative studies indicate that an abundant supply of food material promotes mycelial-mat development, while a substratum less rich in food materials promotes strand and sclerotial development. Sliced healthy carrots and sterilized cottonseed meal were added to soil with diseased-carrot inoculum. In those cultures not invaded by Rhizopus and other fungi the root-rot fungus made an extensive growth of loose mycelium at the expense of strand growth and sclerotial development. In this connection it may be noted that weak corn-meal agar has promoted strand growth, while sterilized corn meal has produced thick mats of mycelium with no individual strands evident. The extension of strands in field soil with its meager food materials is indicated by the behavior of the fungus in the cultures under discussion.

A lessened development of strands was secured, but a more extensive growth of loose wefts of mycelium took place with sterilized soil than with similar non-sterilized soil. In some of these cultures with sterilized soil sclerotia were entirely suppressed, although there was a copious development of loose mycelium. A further study of the food requirements of the fungus is being made which it is hoped may throw further light on the behavior of the parasite in the field.

MOISTURE REQUIREMENTS

The behavior of the fungus in soils of different moisture content was studied by means of these soil cultures. The absolute moisture content for each lot of soil used was determined. It was possible to correlate variations in development of the fungus in different lots of the same soil with differences in moisture content. Tests also were run with the same soil to which were added predetermined amounts of moisture. One such test was set up on August 30, 1929, in which 6 jars were used for each percentage of moisture. The soil and water were thoroughly mixed before the jars were filled. Soil, air-dried to a moisture content of 9 per cent, was used and moisture was added to bring the percentage up to 10, 20, 30, 40, 50, and 60, based on the dry weight of the soil.

In those jars to which 40, 50, and 60 per cent of moisture had been added, growth of the fungus was scant and was confined to the top surface of the soil. Free water collected in all these jars, indicating complete saturation of the soil. Slight growth took place in the jars with 10 and 20 per cent moisture, but it was confined to the vicinity of the carrot inoculum. The most extensive growth of the fungus appeared in the jars of soil with 30 per cent moisture, which indicated that this was the most favorable moisture content for the soil used.

The Houston black-clay surface soil used in the above experiment has been found to have a much higher moisture-holding capacity than the underlying subsoil. It is evident that the optimum moisture content would vary in different soils and would bear a relation to the available water present.

TEMPERATURE REQUIREMENTS

The first sclerotia produced were developed in the laboratory at a period when the mean daily temperatures ranged between 21 and 27° C. Later in the year almost no sclerotial production took place in soil cultures incubated at laboratory temperatures ranging from 5 to 21° C.

Many cultures were run in an incubator at constant temperatures. At 38° C. no sclerotia were formed; a few were formed at 32° C., but a very copious production took place at 27° C. From this preliminary work it is evident that the temperature range favorable for the production of sclerotia in soil cultures of this type lies between 21 and 32° C. It is interesting to note that at depths of 6 to 24 inches, at Substation No. 5, Temple, the soil temperatures fall within this range during the period of the year when root rot is active.

OTHER APPLICATIONS OF SOIL CULTURES

Aside from a study of the factors involved in the development of sclerotia, soil cultures have been used in testing soil disinfectants. Organic mercury, Semesan, mixed with the soil, has inhibited both strand development and sclerotial formation. The cultures have also been used to test the duration of the protective action of various chemicals added to the soil. For this purpose soil from treated plats was collected after the treatment had stood for a period. The soil cultures are adapted also to the laboratory testing of the effect of fertilizers and soil-ameliorating treatments on strand and sclerotial development of the root-rot fungus under field conditions.

SUMMARY

A method is described for culturing *Phymatotrichum omnivorum* in which non-sterilized soil is used as medium and newly-diseased roots as in-

oculum. This type of culture appears to be well adapted to a study of the moisture and temperature requirements for the production of strands and sclerotia in the cotton-root-rot fungus, *P. omnivorum,* under conditions comparable to those found in the field, and to test the effectiveness of chemical and fertilizer applications in cotton-root-rot control.

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THE OCCURRENCE OF VIOLET ROOT ROT IN CENTRAL TEXAS[1]

B. F. Dana and S. E. Wolff

During the summer, fall, and early winter of 1929, violet root rot, caused by *Rhizoctonia crocorum* (Pers.) DC.,[2] was found prevalent in several locations along the courses of the Little and Leon rivers in Bell County, Texas. Preliminary examination indicates that the disease may be generally distributed in the soils along these rivers.

Diseased plants were found in the wooded areas which spread back from the river for a distance of a few rods to half a mile and in the adjoining margins of cultivated fields. The soils of these areas are highly calcareous and vary from clay to clay loam. They are not well drained and are subject to overflow during periods of high water. The vegetation consists of the usual water-course inhabitants. Where the wooded areas are rather wide *Hicoria pecan* Britt. and *Ulmus americana* L. are the dominant species. Societies of shrubs and herbs (Fig. 1, A) are, likewise, pronounced in these areas. Near the stream bed *Salix nigra* Marsh. (Fig. 1, B) is the prevailing species, the individuals of which usually form a dense stand.

The disease did not appear to be selective but was present on nearly all the species of plants growing in the affected areas. All of the species listed below supported mycelium of the fungus, although not all were killed. In many cases part of the root system of a plant was killed, but the remaining part was able to support the individual plant. Only those species clearly parasitized by the fungus are listed in the table. Since only a limited examination of these river courses has been made, it is very possible that more species can be added to the list.

Hosts for Rhizoctonia crocorum

Ambrosia aptera DC.
Diapedium brachiatum (Pursh) Kuntze
Malvaviscus drummondii T. & G.
Melia azedarach L.
Morus sp.
Parthenocissus quinquefolia (L.) Planch.
Phytolacca decandra L.
Rhus radicans L.
Rulac texana (Pax) Small
Salix nigra Marsh.
Sambucus canadensis L.
Smilax bona-nox L.
Solanum elaeagnifolium Cav.
Verbesina virginica L.
Viola missouriensis Greene

[1] Presented at the 1930 meeting of the Southwestern Division of the American Association for the Advancement of Science and published with the approval of the Director as Contribution No. 125, Technical Series, Texas Agricultural Experiment Station.

[2] Specimens of *Helicobasidium purpureum* (Tul.) Pat., the perfect stage of this fungus, have been found in the same area since this article went to press.

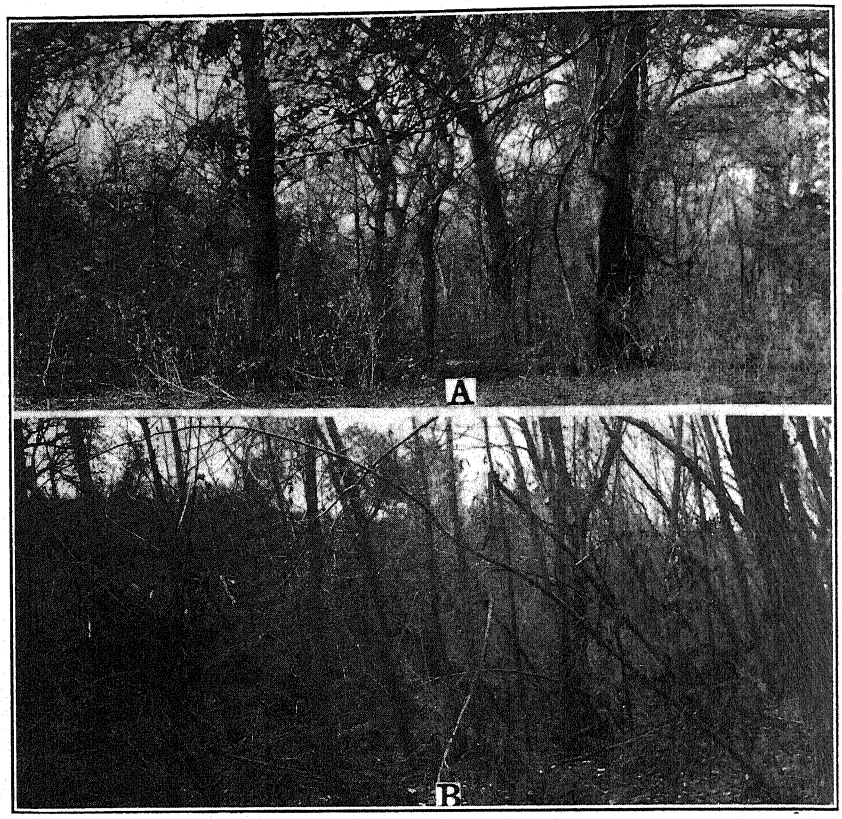


FIG. 1. A. Wooded area along Little River showing a dense layer of partially defoliated American elder, *Sambucus canadensis*, and common pokeberry, *Phytolacca decandra*. Violet root rot was found seriously affecting these and other species in this area. B. Area just above stream bed of Leon River showing dense stand of black willow, *Salix nigra*. Violet root rot was killing roots of willow in the area without causing death of affected trees.

In the areas with abundant moisture the fungus was prevalent on roots in the first 4 to 6 inches of soil. In one dry area at the edge of a cultivated field the fungus was not present on roots near the surface, although the plants had died. On digging to a depth of 16 to 20 inches mycelium was found in abundance. This would indicate that the fungus parasite is favored by ample moisture.

Examination of the Plant Disease Survey records does not show previous reports of the violet root-rot disease in Texas, and specimens of several species sent to the Office of Mycology and Disease Survey were reported to be new records for the United States.

PHYTOPATHOLOGICAL NOTES

Additional data on the range and prevalence of Lima-bean scab.—Mexico is to be added to the known geographic range of scab of the Lima bean, formerly reported[1] on this host only in Cuba and Porto Rico. Such distribution of the disease is shown by a number of specimens of green unshelled Lima beans referred to the writer for verification of the diagnosis by officers of the United States Plant Quarantine and Control Administration and taken by inspectors of the Administration, from Mexican shipments entering the United States at Nogales, Arizona, in January and February, 1931. The shipments were said to be from Caimanero, La Cruz, Rosales, and San Blas, in the department of Sinaloa. On the whole, the lesions on the Mexican specimens are smaller and less mature than on those intercepted from Cuban shipments received in New York during the same period, this year, and are suggestive of the younger lesions on pods from Cuba received early in the shipping season, which began in November. The continued prevalence of the disease in Cuban commercial fields is indicated by the infected pods, from occasional shipments, that have been deposited in the Office of Mycology and Plant Disease Survey of the Bureau of Plant Industry. For reference here it may be stated that during the past fiscal year 2,998,644 pounds of green unshelled Lima beans were imported into the United States from Cuba, 269,627 pounds from Mexico, and a much smaller amount (18 hampers) from Porto Rico.[2] For the present, at least, the pathogene of this disease is identified as *Elsinoe canavaline* Rac.[3] —ANNA E. JENKINS, Bureau of Plant Industry, Washington, D. C.

Color variations in Aplanobacter michiganense.—A white strain of the normally yellow organism, *Aplanobacter michiganense,* the cause of bacterial canker of tomatoes, was reported by the writer in 1929. A second color variant has recently appeared, this time directly from bird's-eye spots on green tomato fruit, identically like those produced by the yellow and white strains of *A. michiganense.* This variant is shell pink, becoming salmon, on Thaxter's potato-dextrose agar; apricot buff on beef agar and potato cylinders. It produces fruit spot and wilt of the tomato typical for *A. michiganense* and is pink on reisolation from such lesions. It is less virulent than the yellow strain; is nonmotile and Gram-positive. Comparative work on the three strains is in progress.—MARY K. BRYAN, Bureau of Plant Industry, Washington, D. C.

[1] Jenkins, A. E. Lima-bean scab caused by Elsinoe. Jour. Agr. Res. 42: 13–23. 1931.

[2] United States Department of Agriculture, Report of the Chief of the Plant Quarantine and Control Administration, August 15, 1930.

[3] *Loc. cit.* See footnote 1.

REPORT OF THE TWENTY-SECOND ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE CLEVELAND MEETING

The American Phytopathological Society held its twenty-second annual meeting from December 30th, 1930, to January 1, 1931, with an attendance of about 200.

The 83 papers delivered before the Society's several sessions may be grouped as follows: General and invitation papers, 4; vegetable diseases, 22; cereal diseases, 20; fruit diseases, 10; tobacco diseases, 6; diseases of miscellaneous crops, 21. Full abstracts of most of the papers presented at this meeting appeared in PHYTOPATHOLOGY for January, 1931.

Two joint sessions were held, one with Section G of the American Association for the Advancement of Science and the other—designated as the Heinrich Anton de Bary (1831–1888) Centenary Memorial program—with the mycological section of the Botanical Society of America. A special session was held on extension work in plant pathology, with special emphasis on methods and agencies used in reaching the people. The last part of the session on tobacco diseases was devoted to discussion of symptoms, diagnosis, and control of these diseases, and several of those in attendance presented specimens, photographs, and stereopticon slides.

Approximately 221 pathologists and their friends assembled for their annual dinner on Tuesday evening. Retiring-President H. S. Fawcett introduced W. H. Weston, Jr., as toastmaster. A selection of songs and several "stunts" were followed by a discussion of the proposed gift to the Imperial Mycological Institute in recognition of the work of E. J. Butler and his associates, by L. R. Jones, Donald Reddick, and others. Motion pictures portraying agricultural activities in the Union of Socialistic Soviet Republics, exhibited by J. G. Dickson, concluded the dinner program.

OFFICERS AND REPRESENTATIVES

The following officers were chosen:

President, M. W. Gardner, Purdue University Agricultural Experiment Station, La Fayette, Ind.

Vice-President, L. M. Massey, Cornell University, Ithaca, N. Y.

Councilor, G. W. Keitt, University of Wisconsin, Madison, Wis.

Associate Editors (three years), Charles Drechsler, U. S. Department of Agriculture, Washington, D. C.; G. L. Peltier, College of Agriculture, University of Nebraska, Lincoln, Nebr.; L. O. Kunkel, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.; and F. D. Heald, State College of Washington, Pullman, Wash.

Business Manager (one year), F. C. Meier, Bureau of Plant Industry, Washington, D. C.

Advertising Manager (one year), J. F. Adams, Agricultural Experiment Station, Newark, Del.

Representatives on the Council of the American Association for the Advancement of Science (one year), D. Reddick, Cornell University, Ithaca, N. Y.; and C. W. Edgerton, Louisiana Agricultural Experiment Station, Baton Rouge, La.

Member of the Board of Governors of the Crop Protection Institute (three years), J. F. Adams, Agricultural Experiment Station, Newark, Del.

Elector to Assist in Naming a Group Representative for Group V, Division of Biology and Agriculture, National Research Council (three years), A. J. Riker, Uni-

versity of Wisconsin, Madison, Wis., alternate, John W. Roberts, U. S. Department of Agriculture, Washington, D. C.; Representative on the Committee of the American Type-Culture Collection (term 3 years, beginning Jan., 1929), C. L. Shear; Representative on Editorial Board of the American Journal of Botany, B. O. Dodge; Representative on the Committee on Cooperation with the Board of Editors of Biological Abstracts, H. P. Barss.

The following temporary committees were appointed to serve throughout the meetings:

Auditing Committee, R. S. Kirby and Donald Folsom.

Committee on Elections, J. S. Wiant, H. T. Cook, and J. A. Trumbower.

Committee on Resolutions, H. W. Anderson, R. E. Smith, and G. W. Keitt.

REPORT OF THE SECRETARY-TREASURER, 1930

The Society now has 747 members in good standing. We have 173 life members—86 paying currently and 87 paid in full—and 574 annual regular members.

During the past year we have lost 58 members and one has been reinstated. Of the 58 lost, 39 have been suspended for nonpayment of dues, 13 have resigned, and 5 have died.

There are 81 applications for new members and, if these are elected, this will bring our membership up to 828. New York leads with 14 applications and Wisconsin follows with 6. It is interesting to note that there are 14 applications from Japan and 3 from India.

STATEMENT OF ACCOUNTS FOR 1930, AS OF DECEMBER 17, 1930

Receipts:

Balance from 1929			$2,702.28
Annual dues: 1925	$ 10.00	(life)	
1926	10.00	(life)	
1927	10.00	(life)	
1928	22.00	($ 10 life)	
1929	41.75		
1930	1,790.22	($260 life)	
1931	2,314.13	($610 life)	
1932	12.40	($ 10 life)	
1933	10.00	(life)	4,220.50
Interest on checking account			44.83
Donations to Lyman Memorial Fund received with dues			11.00
Excess dues			.50
Sales received with checks for dues			2.90
Refund for photos of Des Moines meeting			1.50
Typing list of members for Dr. Schaffnit			4.70
Butler Fund donation received with dues			.50
Total receipts			$6,988.71

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY (1930)	$1,500.00
Transferred to PHYTOPATHOLOGY for Sinking Fund	1,374.00
Secretarial work	543.70
Expenses of Secretary-Treasurer at Des Moines meeting	24.89
Postage and printed stamped envelopes	123.83

Printing (abstracts, menus, letterheads, preliminary announcements, application and nomination blanks, bills)	124.73	
Telephone and telegraph	15.17	
Office supplies	4.27	
Stencils and envelopes for press service	8.55	
Checks returned by bank	25.00	
Charges for collection of checks	1.40	
Donations transferred to Lyman Memorial Fund	6.00	
Donations transferred to Butler Fund ($1.00 not yet dep.)	1.50	
Sales transferred to PHYTOPATHOLOGY	.60	
Photos of Des Moines meeting	1.50	
National Research Council Yearbook	1.00	
Eriksson Prize and fee	50.50	
Balance as shown by bank statement attached	3,182.07	
		$6,988.71

SUPPLEMENTARY STATEMENT

Balance as above stated		$3,182.07	
Plus:			
Estimated income for 1931:			
From annual regular members	$280.00		
From life-sustaining members	27.00		
Interest on checking account	45.00		
	$352.00	352.00	$3,534.07
Less:			
Amount due PHYTOPATHOLOGY:			
For member subscriptions, 1930	766.00		
1931	1,607.30		
Other years	44.92	2,418.22	
Due Sinking Fund ($6.00 per life member):			
1925, 1926, 1927, 1928	24.00		
1930	12.00		
1931[1]	366.00	402.00	2,820.22
Total estimated income for 1931			$ 713.85
Based on 1930 expenses, expenses for 1931 (excluding Sinking Fund and member dues to PHYTOPATHOLOGY) will be			952.50
This would leave a deficit of			$ 238.65

During the past year, of the $10.00 paid by each life member, $6.00 has been transferred to the Sinking Fund and $4.00 to PHYTOPATHOLOGY, leaving none of the income from the life-sustaining members' payments for working funds for the Society. It is evident that a new allotment of funds must be made, both in the case of payments of life members and annual members.

Respectfully submitted,

F. C. MEIER, *Secretary-Treasurer.*

[1] As a result of action of the Council the amount to be credited to the Sinking Fund for 1931 will be $5.00 for each life-sustaining membership payment instead of $6.00 as indicated above.

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1930

STATEMENT OF ACCOUNTS FOR 1930, AS OF DECEMBER 17, 1930

Receipts:

Balance from 1929	$ 487.14	
Subscriptions, 1930	2,503.39	
Subscriptions, 1931	904.29	
Subscriptions, other years	178.79	
Sales of back volumes and numbers	625.16	
Sales, Phytopathological Classics	.42	
Advertising, 1929	98.31	
Advertising, 1930	981.47	
Interest on Sinking Fund	376.75	
Donation from National Academy of Sciences	500.00	
Member subscriptions for 1930 (part)	1,500.00	
Transferred from Am. Phytopath. Soc. for Sinking Fund ($6.00 of each life-sustaining-member payment)	1,374.00	
Principal paid on first-mortgage notes	750.00	
Boyce Thompson Institute, cost of Wilcoxon-McCallan article	192.18	
State College of Washington, cost of cuts in Kienholz-Heald article	31.49	
Member dues received with subscription	5.00	$10,508.39

Expenditures:

Manufacturing PHYTOPATHOLOGY:			
Vol. XIX, No. 12	$528.48		
Vol. XIX, Index	149.47	$ 677.95	
Vol. XX, No. 1	789.84		
No. 2	343.73		
No. 3	371.87		
No. 4	552.84		
No. 5	629.52		
No. 6	340.31		
No. 7	375.04		
No. 8	491.77		
No. 9	401.16		
No. 10	505.60		
No. 11	372.52		
Vol. XX, Cuts	639.25		
Postage	404.32	6,217.77	
		$6,895.72	$6,895.72
Secretarial work			87.75
Expenses of Editor in Chief			81.90
Subscriptions refunded			29.91
Postage			16.66
Expenses of Advertising Manager			173.27
Sinking Fund invested			2,017.17
Mailing lists			8.00
Foreign draft and check returned			63.00
Supplies ($23.85); printing bills ($17.40)			41.25

U. S. Beet Sugar for error in remittance	10.50	
Balance shown by bank statement attached	1,083.26	$10,508.39

Last year our cash balance in the PHYTOPATHOLOGY account was $487.14, this year it is $1,083.26, of which $124.00 belongs to the Sinking Fund. We have paid for eleven numbers of the 1930 Journal and in so doing have not used $766.00 of the amount due from the Society for 1930 member subscriptions. The expenses of the December number and Index will be approximately $750.00 and the commission allowed the Advertising Manager and his secretarial work amounts to $128.57. Thus, a small amount of 1930 expenses will have to come from 1931 receipts, but the amount is much smaller than last year, showing that the financial condition of PHYTOPATHOLOGY is better than it was a year ago.

Much credit is due to Dr. H. B. Humphrey, Editor in Chief, for this improvement in the financial situation of the Journal. By limiting the number of printed pages, cost of publication has been reduced and at the same time a high standard of excellence has been maintained.

SINKING FUND

The Sinking Fund composed of six dollars for each sustaining life membership is now $7,026.00, of which $6,500.00 is invested in first-mortgage notes. Of the remaining $526.00, $402.00 is in the Society's checking account and $124.00 is in PHYTOPATHOLOGY'S checking account.

ENDOWMENT FUND

The endowment fund, established and named for Dr. George Richard Lyman, now totals $1,837.49 in cash donations and pledges, not including 8 pledges of indefinite duration, ranging from $1.00 to $25.00 annually and totaling $75.00 annually. Of this amount, $1,310.49 is placed in a savings account with the McLachlen Banking Corporation. The endowment-fund luncheon, arranged by the Iowa group during the 1929 meeting, added $219.00. During the year one member contributed his first fee received from professional services as a plant pathologist. The large number of member contributors is a convincing expression of faith in the future of PHYTOPATHOLOGY.

STATUS OF SUBSCRIBERS

Our edition of PHYTOPATHOLOGY is 1,625, of which 1,251 copies are mailed to members and subscribers. The number of subscribers in good standing at the close of 1930 is 500, of which 173 are domestic and 327 foreign. The number of subscribers has increased by only two this year. This is largely due to the loss of a number of subscribers from Japan and Russia.

Respectfully submitted,

F. C. MEIER, *Business Manager.*

REPORT OF THE EDITOR IN CHIEF

Throughout the year 1930 the members of the editorial staff of PHYTOPATHOLOGY have sought to improve the general excellence of our Journal. Closer and more critical attention has been given to the quality and scientific interest of the subject matter presented in each manuscript; a more rigid requirement has been set up and maintained governing the character and presentation of illustrative materials accompanying each contribution; and the Journal has held with general consistency to the policy of accepting only those manuscripts that pertain to plant pathology. Papers of a strictly taxonomic nature have been submitted for publication in PHYTOPATHOLOGY but have not been accepted.

The 20th volume of PHYTOPATHOLOGY comprises 1,011 pages of printed matter and illustrations, classified as follows: Eighty-seven articles, 26 phytopathological notes, 3 reports, the constitution of The American Phytopathological Society, 4 book reviews, 119 abstracts, 1 plate, and 185 text figures. From January 1 to December 31, 1930, approximately 131 manuscripts of articles, reports, book reviews, etc., and 86 manuscripts of abstracts were submitted. Of this number, 21 were returned to their authors for revision, 10 were rejected, and 3 were withdrawn by their authors. Of the several manuscripts received during 1930, 20 articles, 2 phytopathological notes, 1 book review, 84 abstracts, and 2 abstracts by title only are in press. About 30 per cent of the papers, exclusive of abstracts, presented in volume 20 of PHYTOPATHOLOGY were contributed solely or in part by employees of the United States Department of Agriculture.

The editorial staff earnestly solicits the cooperation of all, who submit manuscripts for publication in our Journal, in presenting the subject matter of their papers in accordance with a carefully organized outline in which first things come first and in which tables and illustrations are accompanied by suitable and adequate legends. More attention should be given to paragraphing one's subject matter, and the sense of each paragraph should be made immediately available to the reader the moment he has read its first sentence. We must still urge greater care on the part of our contributors in the selection and use of only the very best illustrations. Too many photographs are mere blobs which, when reproduced, show only vaguely or not at all the details the author fondly hopes may be revealed. Another thing we would urge upon our contributors: In all manuscripts accompanied by illustrations, the latter should not be sent loose in an envelope but should be arranged or mounted, by the author, in something like the order in which he wishes them to appear in the published paper. Too much of such detail is left to the editor. Manuscripts in excess of 60 pages are not to be encouraged, though if of exceptional quality longer ones will be accepted.

It is desirable that our journal become more and more truly international in its service. To further its progress in attaining such an aim, contributors who do not write in English are encouraged to submit manuscripts, preferably in German or French, accompanied by a comprehensive resumé in English.

The editor wishes here to express due acknowledgment of his appreciation of the excellent workmanship and spirit of cooperation shown by The Science Press Printing Company in maintaining the mechanical and editorial standards of excellence which characterize our Journal. Greater effort, however, is still necessary if we are to get each month's issue into the mail bags by the first of the month.

Thanks also are due J. Marion Shull for his expert aid in arranging and lettering the illustrative material and to Frances W. Todd for her invaluable clerical and editorial service.

H. B. HUMPHREY, *Editor.*

REPORT OF THE ADVERTISING MANAGER

The advertising returns show a slight increase over those received last year. For the twelve issues of PHYTOPATHOLOGY published during the year 1930, there appeared a total of 119 advertisements distributed as follows: 47 one-page, 43 one-half-page, and 29 one-fourth-page advertisements. The total advertising was equivalent to 75¾ pages, or an increase over last year of 6¾ pages.

The renewal of contracts for 1931 at this time shows a decrease compared with the last five years. Soliciting for renewal of contracts is started in September and there appear to be considerable reductions in advertising budgets for 1931, as indicated by many old-line companies in failing to renew their contracts at this time. This may

be anticipated because of the general business depression and retrenchment. However, this is the time that greater cooperation of members is essential if we expect to maintain a reasonable advertising business. This past year only one member in the entire organization wrote the advertising manager relative to a prospect.

It is suggested that members write the advertising manager of any favorable prospects and, whenever in touch with commercial representatives, mention the opportunity PHYTOPATHOLOGY offers as a practical medium for advertising equipment and materials directly to the members with whom they wish to do business.

J. F. ADAMS, *Advertising Manager.*

COMMITTEE ON INVESTIGATION OF FOREIGN PESTS AND PLANT DISEASES

During the past year this committee has continued its activities in connection with furthering studies on plant pests and diseases in foreign countries. We are fully convinced that such studies by specialists from our own nation constitute the most logical approach to the problems concerned with dangerous parasites that are likely to be introduced at any time from foreign lands.

With the cooperation of Dr. J. H. Faull, we have supplied Dr. Westerdijk at the Phytopathologisch Laboratorium at Baarn, Holland, with species of American elms to be tested for their resistance to the Dutch elm disease and have been informed that our common *Ulmus americana* is very susceptible. Reports on other species will be forthcoming.

Our efforts to secure provisions for the permanent recognition of this work, by the insertion of an item in the Congressional Budget, are being continued in cooperation with the Society of Economic Entomology. We are assured that our recommendations are being given serious consideration and that already the joint resolutions of the organized phytopathologists and entomologists have been influential in the decision of Federal officials to approve the expenditures necessary to send specialists to foreign countries for such special purposes as securing parasites of the Mediterranean fruit fly, the Japanese and Asiatic beetles, Citrus black fly, and the corn borer, as well as for investigations on cereal and other diseases.

As additions to the previous list of dangerous plant diseases which should be investigated, we would add the Mal Secco disease of lemons which occurs in the Mediterranean region, the Bayond disease of the date palm in Morocco and Algeria, and the Elsinoë disease of beans in Cuba, the latter having been intercepted several times at our ports of entry.

C. R. ORTON, *Chairman,*
W. A. MCCUBBIN,
F. D. FROMME.

COMMITTEE ON QUARANTINE AND REGULATORY WORK

1. Your committee would call the attention of this body to the growing need for thoughtful consideration of many important and perplexing problems in the field of State and Federal quarantine and regulatory work and would urge upon the Society as a whole and upon the membership individually a wider and deeper interest in these problems, to the end that we may more completely fulfill our whole professional duty to the public interest.

In this connection the committee ventures to suggest that the Society give due consideration to the policy of including in each year's program some feature dealing with the quarantine and regulatory aspects of plant pathology. And, further, that a place be made from time to time in PHYTOPATHOLOGY for articles of outstanding interest and importance on these matters.

2. The committee presents for your consideration the following resolution:

"This Society believes that the success or failure of State or Federal action against sudden outbreaks of plant disease is often determined by the promtpness with which suppressive or restrictive measures can be fully employed, as well as by the rapidity with which the essential elements of these problems can be ascertained.

"This Society, therefore, declares itself in favor of an 'Emergency policy' involving (1) an emergency fund, protected by suitable safeguards and available only for emergency purposes; (2) arrangements whereby rapid and adequate surveys can be made on short notice; and (3) provision for such immediate research as will develop the information necessary for intelligent action."

We move that this resolution be adopted and that copies of the same be sent to the Secretary of Agriculture, the Plant Quarantine and Control Administration, the National Plant Board, and the Society of Economic Entomologists.

W. A. MCCUBBIN, *Chairman*,
MAX W. GARDNER,
J. F. ADAMS.

COMMITTEE ON EDITING PHYTOPATHOLOGICAL ABSTRACTS

The committee on editing the abstracts submitted for presentation at the annual meeting regards its position as intermediate between two extreme views which have been voiced or tacitly assumed by the Society membership: (1) Some hold that the contributions made by these abstracts are either of minor importance, embodying incomplete or incidental work, or, if they contain really new and significant material, they will later be published in full and the Society is not warranted in standing the expense of duplicate publication; there are also some who attach much less importance to these abstracts than they do to the publication of a complete paper and even take rather lightly the responsibility assumed in making a contribution to scientific literature in this way. (2) Others frankly hold that the Society needs a clearing house, functioning at least annually, for the numerous miscellaneous items that interest the members of the Society during the course of a year's work, so that priority may be promptly established for a novel idea or so that the results of their work may at once be put into circulation.

These questions have been discussed at length and often with warmth in previous meetings of the Society. For the benefit of novitiates of the membership, as well as others who may have forgotten the past, the present arrangement of having all abstracts submitted to careful editorial scrutiny grew out of the arguments for and against the publishing of abstracts at the Toronto meeting in 1922. Because of the extra work involved, a committee is appointed to perform this duty instead of assigning it to the editor of PHYTOPATHOLOGY, but the committee works in close cooperation with the editor.

Out of the experience of reviewing a hundred abstracts each year for the past eight years, this committee has seen the need for correcting certain tendencies in the preparation of the abstracts, such, for example, as tardy submissions; prolixity; ambiguity, especially in titles; and disproportionate use by different members of this privilege of immediate publication. They also have proposed certain rules governing the submission of abstracts which have been voted upon and accepted by the Society. These rules ought to be consulted and observed by all who present abstracts and, beginning with this year, they are being published in the preliminary announcement of the annual meetings.

When, occasionally, it becomes necessary to invoke these rules and reject an abstract or in other ways restrict the privilege of publication, the members concerned should

realize that the action taken is approved by a majority of, and usually by the entire, committee, after consultation with the editor and the secretary of the Society.

Usually only the defects of an abstract, and not all of them, are susceptible to legislative correction; the virtues can be pointed out and extolled but not prescribed for general adoption. The abstracts this year have been the best as regards submission, conformity to the 200-word limit, substance, and manner of presentation, that this committee has seen. It is felt that no further modification of the rules is necessary. However, a few recommendations are offered which may help authors to avoid the sort of defects that can not be corrected by legislation. These follow:

Historical reviews of literature, however pertinent to the new findings, are to be avoided. In nearly all cases an appropriate statement of the new findings will in itself tacitly put these findings into a sufficiently clear relationship to the body of knowledge already extant, pending more complete elaboration in a lengthy paper.

Expressions of opinion as to what constitutes a point of interest, of importance, or of significance, either general or specific, are to be avoided. Efforts at evaluation, while highly appropriate for lengthy publications in which the ramifying implication of a new fact can be adequately dealt with, when attempted in a bold remark or in a number of flattering adjectives, very often convey only a somewhat exaggerated notion of the writer's immodesty. An appropriate statement of new findings should in itself express or imply both the measure of importance and the quality of interest attaching to those findings.

References to work under way but, not at the time of writing, productive of significant results are to be avoided. Even more rigorously to be shunned are references to work not yet begun but contemplated in the future, as well as prophetic remarks concerning the profitableness of this or that line of investigation.

FREEMAN WEISS, *Chairman*,
J. W. ROBERTS,
ANNIE R. GRAVATT,
CHARLES DRECHSLER,
W. A. WHITNEY.

COMMITTEE ON INTERNATIONAL RELATIONS

Following thorough consideration at the Des Moines meeting of plans looking to a closer union of workers in the field of plant pathology, your committee sent copies of the tentative plan, adopted in principle, to as many pathologists as possible in other countries. The response was so cordial that the organizers of the Cambridge meeting of botanists were asked for the privilege of bringing the subject forward for consideration at a formal session of the section My, Mycology and Plant Pathology, of the Fifth International Botanical Congress.

At an informal conference at which at least 50 persons, representing perhaps half as many nations, were present, a discussion similar to that at Des Moines was held in which substantially the same conclusions were reached, namely, that plant pathology should have autonomy within the Botanical Congress and that some more formal international organization than now exists is needed. At the formal session the proposal of The American Phytopathological Society was presented, whereupon three resolutions were adopted, the first two of which were forwarded to the organizers of the Sixth Congress which is to be held in Holland.

1. That at the Sixth International Botanical Congress, plant pathology be given full sectional autonomy.
2. That at the Sixth International Botanical Congress, mycology be given full sectional autonomy.

3. That an international standing committee be appointed to deal with urgent phytopathological needs that may arise between the Fifth and Sixth International Botanical Congresses.

This resolution was adopted in the section My. It was sent to the plenary session of the Congress, proposed by Appel, seconded by E. Van Slogteren, and adopted. The officers of the section My appointed on the standing committee E. J. Butler and Donald Reddick (Recorder) and gave them power to co-opt members.

An international council of plant pathology is thus established to serve a probationary period of 5 years. Obviously, the number and the nature of the problems which are presented to this committee will be instrumental in determining the necessity for its continuance. It is too much to expect that the committee will of its own initiative search for work to do. The problems of international importance which individuals lay before the committee will receive attention from a group duly constituted for that purpose.

A committee designated by The American Phytopathological Society should be continued for the purpose of acting in an advisory capacity to the American representative on the international committee.

R. J. HASKELL, *Chairman*
G. H. COONS,
H. S. FAWCETT,
H. S. JACKSON,
DONALD REDDICK,
J. C. WALKER.

COMMITTEE ON PRESS

Following the plan inaugurated for a more adequate coverage of the Des Moines meetings, the Press Service again prepared paper-by-paper accounts of the Society's several sessions and released to over ninety papers, magazines, and press associations a 29-page mimeographed sheaf of stories based on the abstracts of the meetings.

While the Press Service has no way of determining the use to which the releases were put, information received from time to time indicates that the service has been of value both to the recipients of the material and to the Society. Plans are under way, even at this early date, for the betterment of the material to be released for the coming New Orleans meeting. In this connection, the chairman wishes to express his grateful thanks to Mr. Don B. Reed, day city editor of the *Washington Post*, and other members of journalistic profession for their valuable criticisms and suggestions.

The chairman also wishes to acknowledge his indebtedness to F. C. Meier, Dr. W. J. Zaumeyer, and others for their kind cooperation in assembling and mailing the 1930 releases.

The report published (Science, n. s. 73 (1884): 156–157, 1931) of the twenty-second annual meeting of the Society was prepared by the Press Service.

To the membership of the Society the Press Service makes an urgent plea—not only for itself, but for the Committee on Editing Phytopathological Abstracts (of which, also, the chairman is a member)—for a greater amount of information in submitted abstracts. Those of the past year were the best yet submitted; yet there is room for a vast improvement, for there are still many abstracts from which, to obtain a news story, the Press Service has to draw on its professional knowledge. To fulfill its aim—to present in a form comprehensible to the newspaper reader the facts and discoveries of phytopathology—the cooperation of the Society in preparation of adequate abstracts is requested.

W. A. WHITNEY, *Chairman.*

COMMITTEE ON NECROLOGY

During the calendar year 1929, there was one death not included in the previous report, namely that of Mr. Maturin Livingston Delafield, who died December 18, 1929.

During the calendar year 1930, there have been three deaths as follows:

Dr. William Allen Orton, died January 7, 1930.

Mr. Emil Godfred Arzberger, died January 29, 1930.

Dr. Nathaniel Orson Howard, died September 14, 1930.

A. G. JOHNSON, *Chairman*,
G. P. CLINTON,
M. B. WAITE.

REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

Report of Activities of the Division of Biology and Agriculture of the National Research Council. The present year marks the end of the three-year period during which the writer has served as the representative of Group 5 of the Division of Biology and Agriculture. Group 5 includes The American Phytopathological Society and the Society of American Bacteriologists. During the latter two years your representative has also served on the Executive Committee of the Division. In all, some ten or twelve meetings of the Division and of the Executive Committee have been attended within the three-year period. According to the plan of organization, Group 5 will be represented during the next three-year period by an elector nominated by the Society of American Bacteriologists, while the elector from The American Phytopathological Society will serve as alternate.

The work of the Division is accomplished largely through its committees and affiliated organizations. Some twenty committees have been active during the present year. A complete report of the activities of the Division is prepared by the chairman and is included in the report of the National Research Council. The following items may be noted as of particular interest to The American Phytopathological Society:

The Committee on the Effects of Radiation upon Living Organisms has secured funds from private sources for investigations in this field and during the current year has allotted a total of $22,850 to twenty-three investigators. A very large amount of apparatus also has been secured either as loans or as donations to investigators. A general survey of the field looking toward the development of a broad study of radiation is now under way through seven advisory groups each of which is considered a special division of the field.

The Committee on Infectious Abortion has established a central station at the Michigan State College for the study of the causal organisms of infectious abortion and undulant fever. The work is made possible through gifts from the Commonwealth Fund and from other sources.

A Committee on Microbiology of the Soil has been organized during the year.

Publications resulting from the work of committees include a bulletin of the National Research Council entitled "Weather and Health," which is an outgrowth of the work of the Committee on the Atmosphere and Man; a booklet entitled "Volume, Yield, and Stand Tables for Second-growth Southern Pines," by the U. S. Department of Agriculture, is the outcome of the work of the Committee on Forestry, which also has under way a compilation of the forestry literature of North America, which will include about 50,000 titles; a list of wild drug plants issued by the Committee on Pharmacognosy and Pharmaceutical Botany; and a second edition of "Biological Stains" has been issued by the Commission on the Standardization of Biological Stains. The book entitled "Plant Rusts," by Dr. J. C. Arthur and others, which was sponsored in part

by the Division, has also been published. The preparation of a series of bibliographies in the biological sciences is under consideration by the Director of the Research Information Service of the National Research Council.

Funds available for fellowships in the biological sciences have been increased and the scope has been broadened to include agriculture and forestry. A total of seventy-two appointments has been made for the coming year. Considerable assistance to research in the natural and physical sciences has been available since 1929 through a special fund of $100,000, administered by the National Research Council. A total in excess of $75,000 has so far been distributed in ninety-six separate grants. Of these, sixteen were for projects in the biological sciences. A portion of the fund has been set aside for the support of projects of a general nature which may be utilized for the calling of conferences on specific problems or fields of research.

F. D. FROMME,
Representative of the American Phytopathological Society and the Society of American Bacteriologists.

Committee on American Type Culture Collection.

Number of cultures on hand January 8, 1930	501
Number of cultures on hand December 18, 1930	654
Number of cultures sent to headquarters at Chicago during the year	219

Our facilities for handling cultures have been increased during the year by the addition of a General Electric refrigerator of 13 cubic feet capacity, in which a temperature of 8° C. is maintained. We have also another refrigerator in which a duplicate set of all cultures is stored at a temperature of 5–6° C. Keeping the stock cultures at these low temperatures greatly reduces the labor and expense of transferring them.

C. L. SHEAR,
Representing the American Phytopathological Society.

Tropical Plant Research Foundation. As is, of course, well known to the members of our Society, the Foundation suffered an irreparable loss in the death of its director, Dr. W. A. Orton, on January 7, 1930. This was the more serious, since, at this time, there is much depression in all phases of the plant-production activities of the Latin-American countries. Since this is a part of the world-wide situation, it is impossible to foresee the outcome for the immediate future. One of the very serious phases of this involves the sugar industry, with which the Foundation has had important relations and from which its chief support has been derived. Under the circumstances it has been necessary to await a more stabilized condition in these fields before we can proceed with assurance as to the reliability of the financial support of research projects in connection with organized industry. The Foundation therefore has not, up to this time, considered it expedient to seek to appoint a successor to Dr. Orton. Aside from this, the Foundation has maintained its organization intact and can report the satisfactory continuation of its activities so far as they relate to all projects now in progress.

L. R. JONES,
Representing the American Phytopathological Society.

The American Association for the Advancement of Science. A brief informal report of the representatives on the Council of the AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE was presented by Donald Reddick.

Committee on Chicago "Century of Progress." The American Phytopathological Society, recognizing the unusual significance of Chicago's forthcoming Exposition, "The Century of Progress,"

And assured of the importance of the part which plant pathology—including the study of both cause and control of plant diseases—has played in the general development of plant sciences, and especially in the progress of scientific agriculture.

Authorizes and instructs its official representatives to offer the proper representatives of the Exposition the cooperation and services of this Society in all practical ways to the end that adequate exhibition be made of the progress and present status of this science.

L. R. JONES,
H. W. ANDERSON,
CHAS. GREGORY,
M. B. WAITE,
G. K. K. LINK.

Auditing Committee. We, the undersigned Auditing Committee, have examined the books of The American Phytopathological Society. The books were found in good order.

R. S. KIRBY,
DONALD FOLSOM.

Resolutions Committee. A committee consisting of G. W. Keitt, Ralph E. Smith, and H. W. Anderson, brought in the following report, which was adopted by the Society.

Resolved: That the members of The American Phytopathological Society extend their sincere thanks to the members of the local committee on arrangements, especially to Professor Visscher and Dean Focke of the Case School of Engineering for their efficient efforts in contributing to the success of the Cleveland meeting.

Resolved: That the Society express its appreciation to Western Reserve University for the excellent facilities furnished for the Cleveland meeting of the Society.

Resolved: That the members of The American Phytopathological Society assembled at Cleveland, Ohio, extend to Miss Mary G. Van Meter their very sincere appreciation of her long and efficient service in the interests of the Society and their regrets that she has found it necessary to withdraw from this activity.

Resolved: That provision be made for a standing committee on extension work in plant pathology composed of five members selected by the Council.

ACTION OF THE COUNCIL

In addition to making the appointments mentioned in the first part of this report, the Council made the following recommendations, which were approved by the Society:

1. That, as was the case in 1930, the Editor of PHYTOPATHOLOGY be allowed actual expenses for 1931 up to or within $300.00 for secretarial and editorial assistance.

2. That the summer field trip in southern Indiana and Illinois, scheduled for 1930 but cancelled because of cold injury to fruit trees of that section, be held in 1931, and that the previously appointed committee, consisting of M. W. Gardner, H. W. Anderson, and Leslie Pierce, take charge of arrangements.

3. That the Secretary-Treasurer make allotment of life-membership funds as follows: $1.00 to the Society; $4.00 to the Journal, and $5.00 to the Sinking Fund.

4. In view of the fact that the Secretary-Treasurer has asked to be relieved of the sole responsibility for investment of Society funds, a temporary committee be appointed to advise with the Secretary-Treasurer as to best methods of accomplishing this service and report at the next annual meeting.

5. That the four new associate editors for this year be chosen by the Editor in Chief and Secretary-Treasurer.

OTHER BUSINESS

The Secretary read a letter from T. G. Major, Secretary-Treasurer of the Canadian Phytopathological Society, expressing hope for a successful meeting in Cleveland and naming the officers of that organization for 1931 as follows: President, W. P. Fraser; Vice-President, D. L. Bailey; Secretary-Treasurer, T. J. Major; and Councillors, H. T. Güssow and G. H. Berkeley. Dr. Major extended a cordial invitation to members of our Society to attend the next meeting of the Canadian Society at the Ontario Agricultural College, Guelph, Ont., about June 24 and 25.

The Secretary read a letter from Dr. M. P. Löhnis of Wageningen, Holland, addressed to Dr. L. R. Jones, in which she called attention to the fact that March 15, 1931, marks Dr. Johanna Westerdijk's 25th year as directress of the Laboratory Willie Commelin Scholton. A motion was made by Dr. Jones and carried that, in view of the fact that many of Dr. Westerdijk's friends would wish to write her on this occasion, the Secretary be instructed to provide for the assembling and sending of these letters.[2]

A letter, addressed to the Secretary by Dr. H. M. Quanjer, was read, in which he requested that our members be informed of the fact that the Eriksson Prize has been awarded to Dr. J. H. Craigie; also, that the jury for the virus-disease prize failed to reach a conclusion; consequently, 2,000 Swedish kroner will be reserved for the offering of new prizes at the International Botanical Congress scheduled for 1935 in Amsterdam. Dr. Quanjer expressed the hope that members of our Society will continue to show interest in this international undertaking.

It was moved by Dr. I. E. Melhus, and carried, that the members of the Council consider ways and means of publishing the papers presented in the de Bary Memorial Program as a separate and that, on reaching a decision, they have power to act.

A motion was made and carried that the Council draft a letter to Miss Mary G. Van Meter in recognition of her efficient service to the Society as assistant to the Secretary-Treasurer during the period May, 1922, to August, 1930.

[2] These letters were sent to Dr. Löhnis on February 24 for transmittal to Dr. Westerdijk.

WILLIAM ALLEN ORTON

FEBRUARY 28, 1877—JANUARY 7, 1930

William Allen Orton was graduated from the University of Vermont with the B.S. degree in 1897. He received the M.S. degree at the same institution in 1898, specializing in botany and plant pathology. In 1915 the degree of D.Sc. was conferred upon him also by the University of Vermont.

In June, 1899, Doctor Orton entered the United States Department of Agriculture, two years before the organization of the Bureau of Plant Industry, and for a little more than a quarter of a century was prominently associated in a constructive way with the activities of the Department. During the first decade he carried on researches on diseases that were causing serious losses to cotton, cowpeas, and watermelons. He was a pioneer in the breeding of varieties resistant to the widespread wilt and root-knot diseases of these crops. In 1907 he was made head of the Office of Cotton, Truck and Forage Crop Disease Investigations. Under his direction diseases of the important vegetable and forage crops received major attention. The infectious nature of potato-virus diseases was discovered, and methods of control through the use of certified seed were worked out. He also helped organize the now accepted methods of potato-seed certification. He organized the Plant Disease Survey work and with others started and carried on research on diseases causing losses to vegetables in transit, market, and storage. He was active in the work leading up to the passage of the Plant Quarantine Act, and, from 1912 to 1924, he was Vice Chairman of the Federal Horticultural Board.

He was one of the organizers of The American Phytopathological Society, its president in 1921, and the editor of PHYTOPATHOLOGY from 1916 to 1920, inclusive. He was a member of a large number of botanical, agricultural, and related organizations.

In 1924 he resigned from the Department, after 25 years of service, to become scientific director and general manager of the Tropical Plant Research Foundation and, during the last six years of his life, organized and carried forward important research projects relating to production and diseases of cotton, sugar-cane, chicle, and other tropical and subtropical crops.

Doctor Orton was the author of more than 100 papers, mostly on various phases of plant pathology and on diabetic dietaries. He was a man of unusually broad vision and clear judgment and possessed great original ability for the organization of research. He was always kindly, considerate of others, and of an even temperament; hence his circle of friends was great. In the words of one of his close associates, "He stood on an eminence apparently without realizing his own greatness. He was so friendly, so tolerant, so just, so modest with all, that it seems to me now we worked with a great man without then understanding his true greatness or the rare privilege of serving with him."

EMIL GODFRED ARZBERGER

OCTOBER 3, 1877—JANUARY 29, 1930

Emil Godfred Arzberger graduated from the State Normal School, Whitewater, Wisconsin, in 1903. In 1906, he received the degree of Ph.B. from the University of Wisconsin and, in 1910, he received the degree of M.A. from Washington University, St. Louis, Missouri.

From 1910 to 1913, he was Assistant Botanist at the Ohio Agricultural Experiment Station. During the summer of 1913, he was Research Fellow at the New York Botanical Garden. From the fall of 1913 to the time of his death Mr. Arzberger was Assistant Pathologist, Office of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture.

Mr. Arzberger was a man of high ideals, sincerity, and integrity. He was a tireless, skillful worker in the field of botanical science, which he loved.

NATHANIEL ORSON HOWARD

MAY 11, 1880—SEPTEMBER 14, 1930

Nathaniel O. Howard received the degrees of Ph.B. in 1903, Sc.M. in 1917, and Ph.D. in 1925, all from Brown University. The Rhode Island College of Pharmacy granted him the degree of Pharmaceutical Chemist in 1929.

From 1905 to 1918, Dr. Howard taught chemistry at the Technical High School in Providence, R. I. After 1920 he was, for several years, part-time Instructor in the Department of Botany, Brown University. For several years he also taught botany at the Rhode Island School of Pharmacy. From 1913 to 1917, he worked part time on various problems in forest pathology, in connection with the Office of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture. From 1918 to the time of his death, he was employed practically full time in this connection.

Dr. Howard was of a quiet nature and a kindly disposition, a loyal friend, and cooperative associate, a thorough and beloved teacher, a devoted husband and father, a scientist of broad interests, and a thorough and careful investigator.

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THE METHODS OF CLASSIFICATION OF PLANT VIRUSES, AND AN ATTEMPT TO CLASSIFY AND NAME POTATO VIROSES[1]

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INTRODUCTION

For the viroses of no other plant is the necessity of a logical and internationally understandable classification more urgent than for those of the potato plant. This may be illustrated by the following example:

The common mosaic of the potato variety Bliss Triumph, called "rugose mosaic" in some papers from the Agricultural Experiment Station of the University of Wisconsin (among others, 34), is, according to Johnson (41), not identical with the rugose mosaic of Schultz and Folsom but with their "crinkle mosaic." But, according to Johnson, it is not identical with the "crinkle" of Murphy and McKay nor with that of Quanjer. It, however, resembles more closely the simple or common mosaic of these European authors.

In order to avoid future difficulties of this kind, Johnson attempted to identify and differentiate potato viruses by the determination *in vitro* of certain physical and chemical characteristics which he called the "properties" of the viruses concerned. According to him, the most reliable diagnostic features of the "property" type are the thermal death-point, the longevity *in vitro,* the effect of dilution, and the influence of certain chemicals.

[1] A preliminary sketch of this paper has been presented to the Fifth Botanical Congress, held at Cambridge, August, 1930.

[2] Virus-free material was grown for the writer by Dr. Oortwijn Botjes. Material was sent from abroad by Dr. Cummings, Dr. K. H. Fernow, Dr. Paul A. Murphy, and Dr. E. S. Schultz. In executing the intervarietal transmission experiments, on which are based the results presented, the writer was assisted, in 1927, by Ir. E. Biewinga; in 1928, by Dr. V. Likhité; in 1929, by Ir. C. C. Klijnhout; and in 1930, by Dr. J. H. H. van der Meer. A part of the microscopical work was done by Mr. A. Ovinge. Suggestions or criticisms in the presentation of the results and help in the editing of the English text of this paper were kindly given by Dr. J. Henderson Smith, Dr. Lee M. Hutchins, Dr. James Johnson, Dr. Paul A. Murphy, and Dr. Geo. H. Pethybridge, with whom the writer discussed the subject during and after the Fifth International Botanical Congress. The photographs of figure 4 were made by Ir. K. Leendertz. The writer wishes to acknowledge the valuable suggestions and help of all persons mentioned.

While the determination of these properties offers great possibilities especially for the knowledge of the nature of the viruses concerned and is indeed helpful in clearing up some of the existing confusion, it cannot be expected to give much information on the properties of the viruses which are of primary interest to us, *i.e.*, their properties as pathogenes. It may also be noted in Johnson's work that the same descriptive names that he criticises and that have presented so many difficulties for international as well as for local use—"crinkle mosaic," "rugose mosaic," "leaf-rolling mosaic," and "mild mosaic," for certain diseases; and "wrinkling," "ruffling," "curl," "roll," "stem necrosis," "rugosity," and "streaking" for certain symptoms apparently are indispensable to his report.

The writer, in order to corroborate Johnson's results, has tried to approach the problem from the pathological side. It is evident that the value of the determination of physical and chemical characteristics of the viruses concerned must increase when, at the same time, their properties as pathogenes are elucidated. To this purpose it seemed desirable, first, to give a critical survey of all the methods thus far in use for the identification, differentiation, and classification of viruses and then to choose such methods as appeared to be most appropriate for elucidating the pathological side of the potato-virose problem.

Our knowledge of the virus disease of plants has been gathered by the following methods:

A. Indirect methods (pathological methods according to Quanjer).
   1. Symptomatology.
   2. Morbid anatomy and physiology of the hosts.
   3. Determination of the host range.
   4. Determination of the modes of transmission and of the relation between vectors and viruses.
   5. Determination of the effect of environment on the diseased hosts.

B. Method not yet classifiable as direct or indirect.
   6. Cytology (x-bodies).

C. Direct methods (property methods according to Johnson).
   7. Cultivation of the viruses and determination of their physical and chemical characteristics.

It is not the writer's intention to include in the present paper a survey of the entire literature, nor does he attempt more in the first seven chapters than to give an idea of what already has been accomplished through the use of the above methods and to indicate some additional progress that may be expected to be attained through the further application of these methods to the identification, differentiation, and classification of plant viruses.

In the eighth chapter an attempt will be made to present a classification and nomenclature of some potato viroses on a pathological basis.

CHAPTER 1.

SYMPTOMATOLOGY

That symptomatology plays an important rôle in all studies on virus diseases is evident when we try to determine what virus diseases are. The feature that induces us to combine such different phenomena as infectious chlorosis of abutilon (51), (6), tobacco mosaic (53), stipple streak of the potato (2), peach rosette (81), and alloiophylly of the anemone (43) is not the filterability of the causal agents; neither is it their transmissibility by insect vectors; nor the occurrence of easily visible, amoeboid, intracellular inclusions.

The most generally combining features are the infectiousness, the noncultivability of the viruses *in vitro,* the rapidity with which the viruses are taken up by the living cells once they are introduced into wounds made by insects or other agents, the typical way in which the viruses are spread throughout the plant, and, last but not least, the type of symptoms.

The symptoms differ entirely from those caused by bacteria and fungi, which, as a rule, are much more localized. It is true that from the point of inoculation certain bacteria and fungi may penetrate plant tissues without causing conspicuous injury as the infection progresses, but, upon arriving at a favorable location distant from the point of entry, they may cause local symptoms. When, however, healthy-looking sprouts are cut off from the diseased part of the plant and caused to root in soil or are grafted on a healthy stock, it is, as a rule, possible to grow them free from the rather localized pathogene. In the case of grafting, it is not easy for fungi or bacteria to grow through the uniting callus from scion to stock. In the case of viroses, however, it is almost or entirely impossible to get rid of the disease by cutting off tops or buds and growing them in soil or grafting them on healthy stocks. The virus passes easily from the scion to the stock.

In identification and differentiation, also, symptomatology plays a prominent rôle, since the presence of a certain virus can be determined only by the symptoms it produces after its inoculation. But it is not yet possible to develop a general system of classification on the basis of symptoms. That a special group of "infectious chloroses," for example, may be formed on a basis of resemblance of the group members to the noninfectious chloroses (30, Chapter 12, a. o.) is open to criticism. The fact that the virus, which, in *Abutilon thompsonii,* causes an ornamental variegation, has a necrotic effect on *A. indicum* proves the relation of this group to the so-called mosaics.

That the classification into "mosaics" and "yellows" (18) is also premature will be evident when we think of the great number of viroses, which, according to their clinical picture, could not be grouped under either of these headings: *e.g.,* Klebahn's "alloiophylly" of the anemone and other diseases for which the term alloiophylly seems apt, such as "reversion" or "nettle head" of the black currant (49), the "Quercina type" of *Datura stramonium* (11), the extreme dwarfing of spindling sprout (23) or its reverse, the "giant hill," in potatoes. Besides, the most classical representative of the mosaic diseases, caused by Johnson's tobacco virus No. 1, after being transmitted to *Capsicum annuum,* produces a chlorotic condition without mottling, *i.e.,* a yellows (34). Other critical remarks on the grouping into mosaics and yellows will be made in the following chapter. In spite of these criticisms, a classification into (a) "infectious chloroses," (b) "mosaics and related diseases, such as crinkles, streaks, and topnecrosis," (c) "yellows, leaf-roll, and curly top," and (d) "alloiophyllies" has been provisionally adopted by the writer for teaching purposes.

It must be conceded that one often feels uncertain in deciding on the virus nature of a disease when symptomatology is not corroborated by infection experiments, especially when the diseases in question are not systemic. Single or concentric chlorotic circles or lines on the leaves alternating with normal green tissue, and suggestive of the Liesegang phenomenon, are considered as symptoms of virosis. Examples are a mosaic of pea and one of peony (46) and a tobacco ring spot, all three occurring in the United States, and a disease occurring in the Scotch potato variety Arran Consul and in the Dutch variety Maaike and called "yellow barred" or "chevron marking" by Murphy. This last disease is systemic and graft-transmissible. Priode's tobacco ring-spot virus causes local symptoms in the mature leaf tissue around the point of inoculation, the symptoms usually becoming systemic afterwards; but, "A few instances were observed where systemic infection failed to follow infection" (65). One is inclined to attribute a disease to a virus origin when, as in the case of the concentric-ring necrosis of the potato tuber, characterized by more or less concentric necrotic circles around a lenticel (Fig. 8, C), it cannot be ascribed to a cultivable organism (3). But, in this disease we should then have an example of a virus pathogene entering a tuber from the soil and being restricted to the invaded tuber only, without being subsequently transported through the plant to the new tubers (67).

The writer is strengthened in the view that this disease is of virus nature by the work of Atanasoff (3), who grafted naturally infected tubers to newly dug healthy tubers and observed its development in the healthy potato in a most typical form.

Furthermore, other viroses are known which infect plants from the soil (56). A well-defined example of the restriction of a graft-transmissible disease to one organ of a plant is further given by Hutchins (37) in his work on the phony disease of the peach. He states, in part:

"At this time it became evident that the phony disease is contagious. Inoculations with the juice expressed from the roots or other parts of phony trees have not communicated the disease under the conditions of our experiments. It has thus far been artificially communicated only by means of grafting a phony root to the root system of the normal tree. This behavior places the disease in the peach yellows group, and the infective principle is now regarded as a virus. However, the phony disease differs from peach yellows in that the virus of phony disease has been found only in the roots of diseased trees and does not appear to enter the shoots above the soil line, while the peach yellows virus invades both shoot and root. This explains why the phony disease is not communicated when buds, scions or seeds from diseased trees are employed."

We have to acknowledge the enormous amount of information gained by the method of symptomatology on the viroses of special families. As examples may be mentioned Storey's work on mosaic and streak of sugar cane (87, 88, 89, 90), the studies of Bennett (8) and others on raspberry and of Plakidas (63, 64) on strawberry viroses, the work of Johnson (40) and of Valleau and Johnson (96) on the viroses of tobacco, of Bewley and Corbett (9) and others on those of tomato, and the work of Murphy and McKay (58) and of Schultz and Folsom (77, 78) on potato viroses.

CHAPTER 2.

MORBID ANATOMY AND PHYSIOLOGY OF THE HOST

Many viruses travel through the plant from inoculated leaves to growing points, young developing sprouts, storage places for reserve food, or the vegetative offspring, and sometimes to the sexual offspring, without leaving a trace of their path in the xylem, the intercellular spaces, or the walls of the parenchymatic tissues, as fungi and bacteria generally do. This suggested to several workers that the transport of the viruses and of the products of photosynthesis takes place along the same route. Beijerinck (7) was the first to consider the phloem tissues as the channels of transport of the contagium of tobacco mosaic. Baur (6), by girdling experiments, showed that the infectious agent causing chlorosis in Abutilon travelled down through the cortex or bast. Valeton (95), in his investigation of the sereh disease, found gummosis in the phloem but also in the xylem vessels of the sugar cane. Quanjer discovered phloem necrosis in an early study of potato leaf roll (66).

Later, the observations, seeming to support the view that the phloem and adjacent tissues are related to the distribution of viruses in the plant

became more and more numerous. The galls of the Fiji disease are formed in the phloem (44). Absence of a fibrous sheath and appositional growth at the expense of the phloem strands has been found in Musa attacked by bunchy top (52) and clearing of the veins in *Musa textilis* attacked by a similar disease (60). Atrophy of the phloem vessels is seen in Morus suffering from dwarf (91). Clearing of the veins in the young leaves and phloem necrosis in the whole plant are characteristics of curly top of sugar beets (85). A disturbance resembling phloem necrosis occurs in advanced stages of the American (not the European) beet mosaic (72), (13). Necrosis in the phloem and pericycle is characteristic for curl of Rubus (70) and degeneration in the pericycle for xanthosis of Fragaria (63). Atrophy of xylem and phloem has been observed in *arricciamento* of Vitis (62). Clearing of the veins is the first symptom of some mosaic diseases of Solanaceae (73), (74), (82), and (84). Phloem necrosis occurs in the so-called "zeefvatenziekte" of Liberia coffee (86), the virus nature of which, however, is doubtful. Clearing of the veins also is observed as an early symptom of aster yellows (45).

Perhaps disturbance in the phloem would have been found in more cases had not this side of the problem been neglected by some investigators. Thung (93) proved by a very complete physiological comparison of healthy and of leaf-rolling potato plants that in the latter the accumulation of starch in the leaves can be attributed to neither a deficiency in the respiration nor in the diastatic activity of the mesophyll but only to disturbed translocation. This, in turn, must be a consequence of the pathological condition of the sieve tubes, a condition which ends in phloem necrosis. Accumulation of carbohydrates occurs, not only in potato leaf roll and in the sereh disease of sugar cane (99), mulberry dwarf (91), curly top of sugar beet (16), advanced stages of the American mosaic of sugar beet (72), curl of raspberry (21), *arricciamento* (curl) of Vitis (62), and aster yellows (21), in which at least some abnormalities in the phloem have been found, but also in the spike disease of Santalum (17), the spinach blight (94), peach yellows (10), leaf roll of Vicia (14), and the western or yellow blight of tomato (79), although the five latter diseases seem not to have been sufficiently investigated from a histological standpoint.

Further, in the case of some of the diseases in which abnormalities in the phloem have been found, *e.g.*, Fiji disease of sugar cane, bunchy top of *Musa* sp., xanthosis of Fragaria, and some diseases of the Solanaceae in which clearing of the veins has been found as an early symptom, we either have no knowledge or are insufficiently informed regarding the behavior of carbohydrates and other elaborated food in the leaves.

The histological investigation of different mosaics usually reveals a reduction in length of the palisade cells, a discoloration and reduction in

size of the chloroplasts, and a decrease in the amount of intercellular space in the lighter areas of the leaf. The mosaics are not so readily discernible by histological characters as they are by the pattern of outward symptoms. This seems to be the reason why Hoggan (34) concludes:

"It has not been found possible, as was originally hoped, to establish, to any great extent, any means of classification of viruses on the basis of the microscopical features of the diseased tissues!"

The writer will subsequently show in this paper that, at least for the viroses of the potato, more encouraging results have been reached by the histological method.

From the insufficiency of our knowledge of correlations between histological and physiological phenomena in many viroses it may be concluded that it would be premature to attach a too general significance to the following conclusion of Cook (18):

"These studies indicate that we may have two types of disease; the one generally represented by the various mosaics in which the photosynthetic activities are greatly reduced. The second which is represented by 'peach yellow' and 'little peach,' in which starch is formed in the leaves but not transported to other parts of the plant, the result being that the leaves become hard and leathery."

The same may be said of Dunlap's conclusion (21):

"The mosaic diseases were found to be accompanied by an increase of total nitrogen and by a decrease of total carbohydrates. The yellows diseases were accompanied by a decrease in nitrogen and by an increase in carbohydrate materials, as compared with the amounts of these constituents in normal tissue."

Cook's conclusions have value only for the diseases mentioned by him: sugar-cane mosaic, peach yellows, and little peach.

Those of Dunlap hold good only for tobacco, tomato, pokeweed, cucumber, and raspberry suffering from mosaic and for peach and aster suffering from yellows. A general classification based on their results would be too broad to be of real value.

It should not be inferred from what has been said above that the writer regards the movement through the phloem as the only mode of transport of viruses through the plant. It is thought to be responsible only for the spread over long distances; on the rate of this spread several observations have been made, *e.g.*, by Doolittle (19), who found that the cucumber-mosaic virus spreads through the vigorously growing cucumber plants from the point of inoculation and is present throughout the leaves and stems in four days, and by others whose observations are reviewed by Henderson Smith (32). At least some of the plant viruses can pass

through tissues other than the phloem. For example, tobacco virus No. 1 readily enters the leaves through broken trichomes, is immediately taken up by the living cells, and cannot be removed by washing (36). These facts point to the possibilities that there may be a close association between the virus and the protoplasm and that translocation of the virus may take place through the intercellular protoplasmic connections which are known to extend into and between the cells of the sieve tubes (75). If it is true that these "plasmodesms" form a continuous system throughout the plant, the above hypothesis would give an explanation of the peculiar way in which infection occurs and in which the viruses are spread through the infected plants. This hypothesis will be considered again in the chapter on cytology.

That the method of histological investigation sometimes may reveal mistakes made by a too superficial observation of symptoms or by an indiscriminate use of names will be shown in the last chapter. By this method (histological) it has been possible for the writer to confirm the difference between crinkle mosaic and rugose mosaic as made by Schultz and Folsom (77, 78) through the method of symptomatology and by Johnson (41) through the physical method and to divide into distinct groups some virus diseases collectively known as "streak."

It may be concluded from this rapid survey that much work is left to be done in the domain of morbid anatomy and physiology. Little or no attention has been paid to what happens in the plant during the incubation period; "clearing of the veins" precedes in some diseases the other symptoms, but that a more definite morphological and physiological description of this remarkable phenomenon seems not to be given is rather unsatisfactory.

CHAPTER 3.

DETERMINATION OF THE HOST RANGE

The host range of some plant viruses is very extended, *e.g.*, that of curly top, aster yellows, and cucumber mosaic (80), (45), (20), respectively. Some experiments, however, tending to prove the polyphagous nature of mosaic are open to criticism. Elmer (22) obtained mosaic transmission among species belonging to 15 different families of plants. That a mosaic virus can, as a consequence of transmission by artificial means, be forced to multiply in a given host and to produce mottling does not, however, prove that the mosaics occurring in nature are identical. Van der Meulen (97), employing natural vectors in attempts to determine the host ranges of mosaics occurring in plants belonging to different families, came to the conclusion that the mosaics are as a rule specialized. With the extension of

such experiments over a much wider range of plants, it may possibly be revealed that certain known viruses and supposedly different ones are, in fact, identical or are the same in regard to their causal relationship with abnormalities not yet recognized as viroses.

That the method of transmission in such experiments is of primary importance has been shown by Mogendorff (57). His results with the use of sap for the artificial transmission of cucumber mosaic to tomato were inconsistent and the percentage of infection was variable. Occasionally symptoms of "fern leaf" appeared, but they were not very typical. In most cases stunting and chlorosis were produced, sometimes accompanied by a faint mottling. Not until Hoggan (35) succeeded in transmitting cucumber mosaic from tobacco to tomato with the peach aphid did Mogendorff find that by means of this aphid, instead of using sap, he could transfer cucumber mosaic via *Nicotiana rustica* to tomato and obtain a high percentage of infection, with nearly all of the infected plants showing marked fern leaf.

The host-range method has been especially valuable for the discovery of "carriers," or plants in which a virose remains "latent." Such carriers have been detected in the genera Saccharum, Humulus, Beta, Abutilon, Phaseolus, Nicotiana, Physalis, and Solanum.

The value of differential hosts for identification work is limited by the fact that the pathogenicity of a virus may be decreased or increased by some of them. Carsner (15) and Lackey (47, 48) found not only that some weeds are difficult to infect with the curly-top virus but that the virus is "attenuated" by passage through them. The writer observed that a virulent acropetal necrosis of the potato was attenuated by passage through a seedling which behaved as carrier.

An example of what may be called increased virulence has been described by Johnson (39). The percentage of infection of a virus occurring in apparently healthy American potatoes was increased, the incubation period shortened, and the host symptoms accentuated, through successive cotton-needle transfers through tobacco, after which this virus was even capable of causing a serious necrotic disease when inoculated back into the variety of potato from which it was first extracted. Smith (82, 84) described a case of what seems to be the increase in virulence of a potato-mosaic virus. This virus, by needle-inoculation into tobacco, was induced to cause a ring spot in the latter plant and when brought back to potato, from which it was originally extracted, was induced to cause an intensified and killing mosaic. The possibility, however, may be considered that the mosaic potato plants of Smith contained two viruses. This consideration is based on the following facts. When scions of apparently healthy Zee-

land Blue potato plants are grafted on either of the varieties Duke of York or Green Mountain acropetal necrosis develops in the latter. When, however, parts of apparently healthy Duke of York or Green Mountain (which are carriers of the top-necrosis virus described in the last chapter of this paper) are grafted onto apparently healthy Zeeland Blue potato plants a mosaic develops in the grafted plants. It is clear that the grafted plants of this variety contain two viruses but show only mosaic. The method of differential hosts is, in some cases, apt for the analysis of such a virus complex. From the Zeeland Blue described above, virus causing acropetal necrosis can be brought into evidence by grafting on one of the potato varieties which is a regular carrier for top necrosis, and the topnecrosis can be made visible by grafting on one of the varieties in which the acropetal necrosis remains latent.

CHAPTER 4.

DETERMINATION OF THE MODES OF TRANSMISSION AND OF THE RELATION BETWEEN VECTORS AND VIRUSES

It is a remarkable fact and suggestive for further investigation that, as far as our knowledge goes, the groups of virus diseases mentioned in the first chapter are specialized in their modes of transmission. The diseases of group (a), infectious chloroses, are transmissible by grafting only. Those of group (b), mosaics and related diseases, are transmissible by grafting, as well as by sap and by insects. Those of group (c), yellows, etc., are, as a rule, transmissible only by grafting and by insects, whereas the modes of transmission of the members of group (d), alloiophyllies, are insufficiently known.

Some exceptions may be mentioned in group (b), mosaics. Neither the American nor the European sugar-beet mosaic seems to be easily transmissible with juice (72), (13). As exceptions in group (c), may be mentioned the following: "peach yellows" and "sandal spike," neither of which has thus far been possible to transmit by insects. Without any doubt, a more detailed study will reveal a greater number of exceptions and it may be possible also that group (a), infectious chloroses, will lose its isolated place.

It is clear that viroses transmissible by juice are separable from those transmissible solely by grafting or by insects and that both groups are separable from those transmissible solely by grafting. When a host contains two viruses, each of which has a strict relation to a different insect, separation is possible. An insect then may be used to isolate a virus from a juice- or graft-transmissible complex.

Severin (79) worked with tomatoes, which were suffering from both mosaic and yellows. In the tops of the shoots only the mosaic was visible.

He cut off these tops and placed them with the basal part in water and protected them by insect-gauze from the attacks of insects. Then virus-free males of *Eutettix tenellus* were allowed to feed on these shoots and were afterwards transmitted to healthy sugar beets. The latter were infected by the insect—which behavior gave evidence of the fact not only that the curly-top virus—which by previous experiments had appeared to be identical with the tomato-yellow virus—was present in the tops of the original tomatoes, but also that it is possible to use an insect adapted to a certain virus as a means of isolating this virus from a complex.

It is not necessary to dwell here on the relations between insects and viruses, which have been excellently surveyed in Kunkel's conspectus of virus diseases of plants (46). Elze (23) has used these relations as a means of classification. His recent work on this subject is presented in a paper in the current number of this journal.

Elze also showed that it would be a mistake, made by some investigators, to suspect insects that have been found in large quantities on a certain host to be the vectors of a virose attacking this host. Although leaf hoppers can often be found in great numbers on potato plants, they have no significance in the spread of leaf roll. Some spring migrants of *Myzus persicae* are sufficient to spread this disease in the beginning of the summer. They visit several plants in succession, depositing some young on each plant. Although, on the isle of Java, *Aphis sacchari* is much more common on the sugar cane than *A. maydis*, only the latter is a vector of sugar-cane mosaic.

This chapter may be concluded with the discussion of another example of what possibly may be regarded as the selection by the aphid *Myzus persicae* of one virus from a virus complex. Smith, in his experiments mentioned in the previous chapter, inoculated healthy tobacco with sap from a mosaic potato in two different ways, *i.e.*, by needle and by aphid. In the former case he obtained a ring spot, and in the latter a characteristic clearing of the veins developed as the first symptom on the young leaves, followed by the appearance of dark green lines along the veins, a phenomenon resembling what is called "vein banding" by Valleau and Johnson (96). Apart from increase in virulence, each of these diseases kept its own character in successive transfers through tobacco or back to potato, the former producing always a necrotic and lethal effect. In his first paper on this subject, Smith (82) believed that the mosaic potato contained only one virus. In his second paper, he concludes:

"It would appear that the aphis does not transmit some element of the virus which is concerned with the production of the more necrotic and lethal symptoms."

A third assumption would be that his original potato plant contained two viruses and that only one of these was transmitted by the aphis, whereas both are adapted for mechanical transfer.

The mosaic Zeeland Blue, mentioned in the end of the third chapter, shows a marked analogy with the mosaic potato plant experimented with by Smith; its virus, causing acropetal necrosis, is easily transferable through *Myzus persicae,* whereas thus far it seems impossible to transmit the top-necrosis virus by the aphid.

CHAPTER 5.

DETERMINATION OF THE EFFECT OF ENVIRONMENT ON THE DISEASED HOSTS

One general feature seems to hold good for all virus diseases, namely, that infection is independent of the presence of moisture. This may be attributed to the fact that the viruses are immediately taken up by the protoplasts as soon as insects or other agencies introduce them into the cells.

It is a well-known fact that the symptoms of some viroses do not develop at low temperature, *e.g.,* acropetal necrosis of potato (2) and tobacco mosaic in potato (12), while those of others are masked by high temperatures, *e.g.,* some potato mosaics (29). Top necrosis resembles the description of yellow dwarf made by Barrus and Chupp (5), but the fact that the latter disease does not show up in the cool summer climate of Holland proves that they are not identical.

Also, the manifestation and type of symptoms may be affected to some extent by external conditions, as was shown by Henderson Smith (31) in the case of a potato mosaic in tomato, where temperatures above 70° F. markedly reduced necrotic spotting and favored mottling.

Since the thermal conditions in the interior of a plant are to a certain extent independent of environment, such phenomena cannot be looked upon as due to the action of temperature on the virus; they must be dominantly due to its influence on the host.

Smith observed that temperatures above 80° F. masked the symptoms of the intensified potato mosaic, which, in his previously mentioned experiments, were obtained in potato after a transfer through tobacco and that the same temperatures did not affect the ring spot, *i.e.,* the form the disease takes in needle-inoculated tobacco. This would appear to be an example of the common experience that optimum conditions for the plant are optimum conditions for the appearance of symptoms. In looking over the details of Smith's experiments, one finds that not only is the needle-introduced ring spot in tobacco favored by high temperature but that the

aphis-introduced vein banding is likewise favored. This fact may have led Smith to his interpretation that one virus is responsible for both phenomena. If Smith is correct in this, we would have here an example of the influence of the means of transfer on the symptoms, showing some analogy with the result arrived at by Mogendorff and treated in the chapter on the determination of the host range. It is clear, however, that the question whether the mosaic plants from which his experiments were started contained one or two viruses cannot be solved by environmental studies and that we may anticipate that new experiments with differential hosts or differential treatment with physical or chemical agencies will throw further light on this problem.

CHAPTER 6.

CYTOLOGY

A survey of what has been learned from cytological investigations about the amoeboid, vacuolate intracellular inclusions, commonly called x-bodies, has been given (1) by Kunkel (46) and Likhité (50), who, along with Goldstein (27, 28) and others, consider these bodies to be stages in the life cycles of virus parasites, and (2) by Hoggan (34) and Henderson Smith (33), who, as did Iwanowski (38), the discoverer of the bodies, and as do most workers, incline to the view that these bodies are to be interpreted as products of the reaction of the infected cell to the presence of the virus.

It would be to no purpose to repeat here the arguments of the two groups; however, one remark may be made in favor of the latter. When we see how readily in irritated cells solids are precipitated and condensed on the starch grains, for example, the conception that the x-bodies may be inanimate by-products of the protoplast seems to be more probable. This hypothesis is supported by the work of Kerling (42), who, using Guilliermond's method, stained living cells of potato tubers with neutral red and found that the cell vacuole alone first takes the stain, the remainder of the protoplast being unstained. As soon, however, as the protoplast is damaged, dispersion of the stain becomes general in the cell and a solid, red substance from the stained protoplasm condenses on the surfaces of the starch grains.

If we consider the viruses as extremely small organisms living in the protoplast and transported, as was intimated in the foregoing, by the cytoplasmic connections, it is hardly possible to imagine their sudden growth into x-bodies. That these x-bodies may be centers of condensation products of the protoplast is a more plausible interpretation and is substantiated by the observations of Henderson Smith (33).

Carriers offer a possible answer to this question. When a virus causing symptoms in one host variety and remaining latent in another appears to

be associated with x-bodies in the former and not in the latter, it would seem that these intracellular bodies are a result rather than a cause of the disease.

In her investigations on the viroses of tobacco and potato and on cucumber mosaic in solanaceous hosts, Hoggan showed that the presence or absence of vacuolate bodies, and in some cases the combined presence of vacuolate bodies and striate material have undoubted value for the identification of certain viroses. The tobacco viroses in which striated material occurs were further shown to be closely related by the similar behavior of the viruses when subjected to varying intensities of application of physical agencies, such as heat (Johnson's method).

The "bodies," whose presence characterized a certain virose in one host were nearly, without exception, found to develop in other hosts to which the virus was transferred. Since only vacuolate material or vacuolate bodies and no striate material were found associated with the potato viroses studied by Hoggan, it may be concluded that these viroses are not identical with the three tobacco viroses in which striated material occurs.

It seems, however, that neither shape nor dimensions nor structure of the vacuolate bodies is of diagnostic value. These bodies are all more or less similar in almost all the viroses in which they have been found.

No vacuolate bodies were found in the cells of certain Green Mountain potato plants called "healthy," but that these plants were actually virus carriers was demonstrated when top necrosis developed in plants of Dutch potato varieties bearing grafts from the Green Mountain healthy plants. Cytological investigations in connection with this behavior may be looked forward to with great interest.

CHAPTER 7.

CULTIVATION AND DETERMINATION OF PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE VIRUSES

Symptoms in viroses may be regarded as reactions of the host to the presence of one or more viruses and are therefore influenced by factors that pertain to the host, such as species, variety, age, and environment. This and other reasons have induced workers to determine the characteristics and proprieties of the viruses outside of the plant and to express them in terms of mechanical measurements whenever possible. To this end the methods of cultivation, filtration, dilution, aging, inactivation by heat, x-rays, ultraviolet light, and chemicals have thus far been applied.

Johnson is convinced that the plant viruses have properties of specificity and stability comparable with those of other organisms. The viruses expressed from plants are, however, associated with many other substances, and no one has succeeded in getting rid of these materials by pure culture

of the viruses *in vitro*. It would be to no purpose to mention here the efforts made by different workers to get by filtration, centrifugation, or chemicals, viruses of absolute purity and in a controllable concentration. It is hoped that methods are being developed and will be further developed by which it may be possible in the future to determine physical and other characteristics of viruses *in vitro* independently of uncontrollable variables.

According to Walker (98), a common virus causing the mosaics of cucumber, tomato, and Physalis has not the same properties in juice pressed from infected tomato or ground cherry as in juice pressed from infected cucumber. Extracted from the former two hosts it has, just as the tobacco mosaic, a high resistance to aging, heating, dilution, and treatment with alcohol. Extracted from the latter host, this resistance is considerably decreased.

As has already been pointed out in the foregoing, it would be a misconception to think that there is only one mosaic virus the character of which is changed by each of the hosts in which it lives. It is not surprising that juices of certain plants are toxic to the juice-borne viruses of other plants *in vitro*. Indeed, McKinney (55) proved that the juice of healthy cucumber has a very marked effect in rendering the virus of tobacco mosaic more susceptible to thermal inactivation. That the thermal resistance of cucumber-mosaic virus was increased by a passage through Solanaceous plants needs confirmation by an experiment with real cucumber mosaic. According to Johnson (40), the virus of cucumber mosaic is very different from all the 9 viruses designated by him as tobacco viruses. It differs from them in the symptoms it produces in some standard hosts belonging, respectively, to the Solanaceae, Cucurbitaceae, and Phytolaccaceae. Each of the viruses he worked with produces different symptoms in one or more of these hosts, and these viruses differ also in one or more of the following characteristics: resistance to aging *in vitro*, thermal death point, and resistance to the killing effect of 50 or 60 per cent alcohol and of 0.5 per cent nitric acid.

In some cases it is possible to analyze virus complexes by physical or chemical attack.

Henderson Smith (31) has, from a combination disease in tomato, inactivated the potato-mosaic factor by treatment of the tomato juice with 90 per cent alcohol for one hour and retained the other factor, a virus causing yellow mosaic.

It may be that the question whether Kenneth Smith (82, 84) worked with one or two viruses in his experiments can be solved by attacking the leaves of the tobacco suffering from the needle-induced ring spot by heat, by chemicals, or by drying. According to Valleau and Johnson (96), the American "healthy-potato virus" (probably the top-necrosis virus is intended) does not withstand drying, and by this means they have eliminated this virus from a complex which caused streak in tomatoes.

It should be borne in mind, however, that a classification of all viruses on the basis of their physical and chemical characteristics may never be possible on account of the fact that a great number of them are not transmissible with juice under present methods of experimentation. It is conceivable, of course, that new methods may be devised which would obviate these difficulties.

CHAPTER 8.

A CONTRIBUTION TO THE CLASSIFICATION OF POTATO VIROSES

As a general conclusion from the foregoing it may be stated that present knowledge is insufficient for the classification of plant viruses but that the correlation of different methods offers possibilities. Further, it appeared that the morbid anatomy and physiology of the host is a more or less neglected phase in the study of plant viroses. The study of potato leaf roll, however, shows that by correlation of the histological and physiological methods a better conception of the pathological process can be obtained. The reduced growth of the plant suffering from leaf roll, the stiffness of its leaves, the accumulation of the products of photosynthesis in them, the shortness of its stolons, and the reduction of the yield of tubers could be attributed to disturbed transport; this, in turn, to a diseased condition of the sieve tubes which ends in phloem necrosis. It is the aphid *Myzus persicae* which introduces the virus in the sieve tubes.

At the International Congress of Plant Sciences held at Ithaca in 1926, a Committee for the nomenclature of potato viruses was formed. As a consequence, a standard named collection of American potato-virus material was received by the writer through the courtesy of Dr. Schultz and Dr. Fernow. Previously, a disease resembling what Murphy has called crinkle and what Schultz and Folsom have called rugose mosaic had been isolated by the writer from the old American Cowhorn potato, received from Dr. Cummings. Dr. Murphy, of the Irish Free State, was also kind enough to supply some virus types.

Not only did this rich collection of material give the opportunity of comparing the ever-increasing types of mosaic, crinkle, and streak from the Old and the New World but also a new impetus was given attempts to correlate outward symptoms with phenomena of morbid anatomy.

The viruses from abroad have been transferred through Murphy's method of core grafting on Dutch potato varieties. The present communication will be restricted to the results obtained through this method with some of the potato viroses occurring in America and Europe. Other viroses, *e.g.*, witches'-broom and spindle tuber have not yet been sufficiently investigated.

The discovery that all American varieties, whether supposedly healthy or known to be diseased, are carriers of a virus causing necrosis in certain

Dutch varieties and the comparison of it with another necrotic disease for which certain Dutch potato varieties are carriers led to the differentiation of the virus diseases collectively known as streak into two groups, top necrosis (Lat. acro-necrosis) and acropetal necrosis.

Schultz (76), who must have observed the first of these two diseases after inoculating healthy plants of Dutch potato varieties with inoculum from healthy American varieties, says:

"It appears necessary to assume that the necrosis in question is not the so-called 'streak,' since evidence has shown that the Green Mountain as well as other American varieties used in this investigation is susceptible to this malady."

It appeared justifiable to introduce the name top necrosis for this new disease, and this actually has been done in a Dutch publication (Quanjer and Oortwijn Botjes, 69). And it appears still more justifiable to do this, although for international use acro-necrosis is better suited, since it was found that an internal necrosis radiates from some internal phloem strands into the surrounding tissues, killing the tender tips and extending downward through veins, stems, petioles, and tubers, and killing sometimes the apical eyes of the tubers, also.

The second disease, which came into evidence when healthy-looking plants of some old Dutch varieties were grafted on Green Mountain, is identical with a disease described as streak by Artschwager. According to the latter investigator, it is evident that the lesions referred to as streak are elongated and that "their advance along the stem is acropetal." He continues:

"The lesions are the result of necrotic changes in the cells of the collenchyma and adjacent tissues" and "in advanced lesions the necrotic areas extend through cortex and vascular tissues into the pith."

The writer feels obliged to credit Artschwager (1) with the introduction of a sound pathological concept instead of the often-misused term, streak, and proposes the name acropetal necrosis for the disease characterized by a necrosis beginning in the collenchyma, extending in severe cases centripetally, and advancing acropetally along the stem. There are several related diseases belonging to this type described farther on in this paper.

A further possibility of founding classification and nomenclature on a sound basis is opened by the fact that different potato varieties react differently to the same virus. A virus remaining latent or producing a mosaic in some varieties may cause a well-defined necrotic effect in others. In so far as the writer has followed the matter, he is of the opinion that a certain mosaic of the Zeeland Blue potato produces top necrosis in Paul Krüger and that some mosaics which resemble more closely the rugose type

of Schultz and Folsom produce collenchyma necrosis and belong to the acropetal-necrosis group.

In order to clear the ever-increasing mosaic group, the principle should be followed that a potato virus be named after the histological symptoms it causes in the variety reacting most typically in this respect.

As a consequence of this principle, the names rugose mosaic and crinkle must disappear at least as names of sections, for it seems that all types of rugose mosaic and the greater part of crinkle, when grafted onto certain potato varieties, behave as types of acropetal necrosis. Only one case of mild mosaic (the mosaic in Zeeland Blue mentioned above), after being transmitted to Paul Krüger and other varieties, appeared to produce top necrosis.

Most of what hitherto has been mentioned as mild, intermediate crinkle and interveinal mosaic is here provisionally still classed in the section of anecrotic mosaics; but is may be possible to produce necrosis also with some of these mosaics on certain varieties. That being so, the result of the system now proposed will be to exclude a number of potato mosaics from the group of anecrotic mosaics, just as a *fungus imperfectus* must be classed under the Ascomycetes as soon as an ascigerous form appears to be associated with it.

A disadvantage of this method is that the progeny of a variety suffering from top necrosis or acropetal necrosis has very often such an extended necrosis that differentiation is no longer possible. The disadvantage, however, of basing a classification on the varieties which react with mosaic symptoms will be considered by investigators as still more important, knowing how unsatisfactory it has been in the past to describe an ever-increasing number of slightly differing mosaics.

It is proposed here that the Dutch variety Paul Krüger, called President, in England, be used as a standard variety in other countries as well, since it shows up and differentiates all the viroses with which the present writer has worked. It has rendered excellent service in differentiating acropetal necrosis and top necrosis. A disadvantage is that it behaves as a carrier towards para-crinkle, described by Salaman and Le Pelley (74). For the identification of this disease the variety Arran Victory is needed. Since all the American material appeared to consist of carriers of top necrosis, the comparison of American potato viroses other than top necrosis with Europeon viroses can take place only on varieties that mask this disease, *e.g.*, Green Mountain or Duke of York. Varieties that mask acropetal necrosis are needed for the identification of other viroses which occur in the carriers of this disease. The present state of the classification work at Wageningen is indicated in the following pages, which may be taken as a progress report.

SECTION I: ANECROTIC MOSAIC

No necrosis, no streak, no drop of the lower leaves of other varieties after they have been grafted with the virus-carrying scion; only mottling and more or less wrinkling of leaflets. Some viruses, after being inoculated into different potato varieties, have thus far produced only mild mosaic, intermediate mosaic, crinkle mosaic, or interveinal mosaic. They are both sap- and aphis-transmissible (77, 78) (23), and they have a lower thermal death point than does a virus causing crinkle or rugose mosaic (41). Masking at high temperatures occurs in this group. Vacuolate material and bodies have been found especially in these mosaics; striate material is not present (34).

In case one or more of these viruses should be found as a result of later work to produce a necrotic disease in some host variety it ought, according to this system, to be classed under one of the groups of necrosis.

Aucuba mosaic in potato differs from the viroses mentioned above in that there is no wrinkling at all in the diseased plants and that it is not transmitted by aphids, or only exceptionally so (23, 68).

SECTION II: PHLOEM NECROSIS

Necrosis restricted to the phloem strands, i.e., sieve tubes and companion cells; strong accumulation of carbohydrates in the leaves. This necrosis is only visible with the high-power objectives (enlargement 100 or more). In the plants infected in the current season, the necrosis is faint and can be detected in some sieve strands only; in the progeny of such plants "perpetuation" symptoms appear and the necrosis is distinct in a much greater number, generally more than half, of the intraxylary and extraxylary phloem strands (compare the necrotic phloem strands in figure 1, B, with the healthy phloem strands in figure 1, A).

The virus causing phloem necrosis thus far appears not to be sap-transmissible. It can be transmitted by grafting; and, in nature, is transmitted by *Myzus persicae* (61) (77, 78) (23) (58), and (83).

The accumulation of carbohydrates is a consequence of the pathological condition of the sieve tubes, and this condition ends in phloem necrosis (66) and (93). No x-bodies have been found (34).

In European potato varieties, the necrosis appears in the phloem of the foliage and stems, only; but in the American variety Green mountain it also occurs in the phloem of the tubers (Figs. 1, C, and 8, A) of the current season where it is visible to the naked eye (net necrosis of Gilbert, 26). That the American leaf-roll virus is identical with the European was proved by Elze and Quanjer (24).

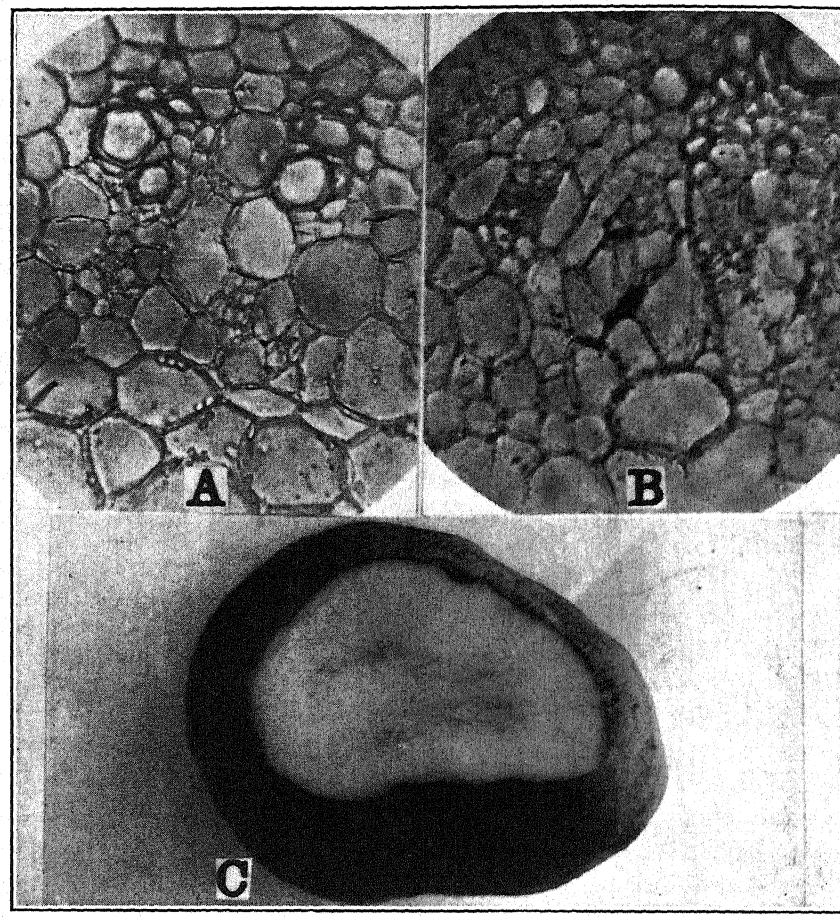


FIG. 1. A. Normal phloem as it occurs in healthy potato plants or plants attacked by anecrotic mosaics. Two internal phloem strands are seen in cross section, surrounded by pith cells and, at the periphery, by some xylem vessels. B. In the midst a necrotic phloem strand typical of potato plants suffering from leaf roll. The necrosis is restricted to the sieve tubes and companion cells. It occurs in the internal as well as in the external phloem. C. Tuber of American potato variety (Green Mountain) taken from a plant, infected by leaf roll from a European variety (net necrosis of Gilbert, 26).

SECTION III: TOP NECROSIS (ACRONECROSIS, LAT.)

Necrosis radiating from only a rather small percentage of the internal phloem strands, almost never from the external phloem strands, into the surrounding parenchyma, this in turn surrounded by a cork cambium, except in the tender tips, which are soon killed; occurring in foliage, stem, and tubers. In the tender leafy tips an internal necrosis appears and is

at first localized in certain phloem strands inside the xylem, from which centers it soon progresses radially in the surrounding parenchyma and appears at the surface. The young leaves of the tips then develop necrotic spots (Fig. 4, A). These necrotic spots of leaves, petioles, and apical stem end later coalesce to the extent that the tender tips of the shoots are entirely killed (Figs. 5, A, and 6, A).

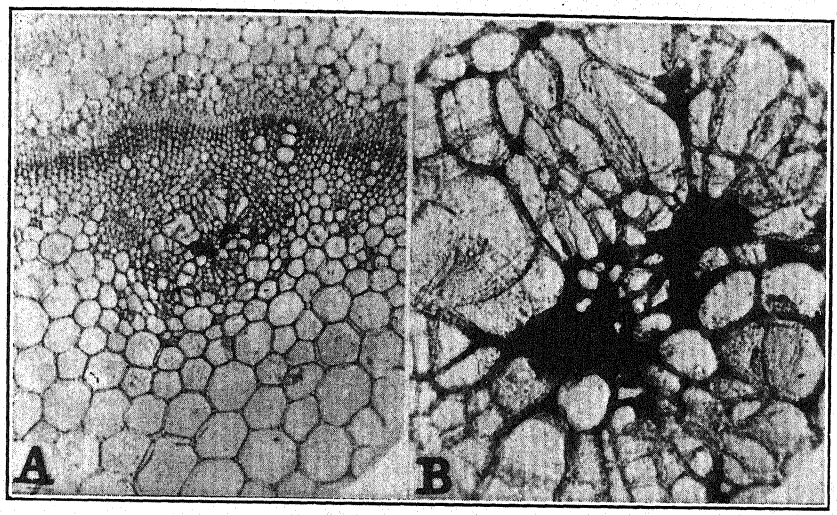


FIG. 2. A. Necrosis radiating from the internal phloem in the surrounding parenchyma as it occurs in some Dutch, German, and English potato varieties that have been grafted with scions of apparently healthy plants of the American varieties Green Mountain, Irish Cobbler, and Rose 4, the Scotch variety Duke of York, and the French variety Jaune d'Or. This necrosis is the histological characteristic of the disease called top necrosis or acronecrosis in this paper. B. Portion of A enlarged. By the formation of cross walls a cork cambium of flat cells is being formed that separates the necrotic cells from those still sound.

Immediately below the dead tips the strands of necrotic tissue consisting of phloem and adjacent parenchyma cells are surrounded by a layer of cork cells. In figure 2 it can be seen that by the formation of cross walls a cork cambium of flat cells has arisen. In the leaves directly below the affected tips, necrotic spots, only, are found; the leaves still lower down remain free from spots. Although the phloem necrosis found in plants suffering from leaf roll is restricted to the sieve tubes and companion cells and can be seen only with high-power objectives, the phenomenon described here can be seen with a pocket lens and, in the tubers, it is plainly visible to the naked eye, appearing as large blotches. In the tubers, also, it starts from the internal phloem and sometimes the apical eye is killed (Fig. 7, A).

The top-necrosis virus is sap-transmissible; but, whether it is transmissible also by *Myzus persicae* is doubtful. Thus far nothing has been seen

which points to the possibility of transmission by insects (*cf.* Elze's paper, in this number of PHYTOPATHOLOGY).

No x-bodies have been found by Hoggan in American healthy potatoes carrying this virus.

After grafting scions of apparently healthy Green Mountain, Irish Cobbler, Rose 4 (American), Duke of York (Scotch), and Jaune d'Or (French) onto several Dutch and German varieties, top necrosis showed up in the grafted plants. The apparently healthy scions used in this experiment were carriers of a virus that produced in both the Dutch and the German varieties a disease that had never before been observed in Holland under field conditions. (It has, however, been observed by the writer in the "massif central" of France.)

Top necrosis may be caused by more than one virus (69). For the purposes of this discussion, the group as now known may be divided tentatively into two types, A and B. This division is somewhat suggestive of the classification of the rusts into biotypes.

Type A includes: (1) A virus that is latent in the Scotch variety Duke of York and is semilatent in Zeeland Blue, causing a mosaic in this variety; (2) a virus that may be identical with (1) but is known to be latent in the American varieties Green Mountain, Irish Cobbler, and Rose 4; and (3) a virus recently found to be carried by the French variety Jaune d'Or and which may possibly be identical with (1) and (2) of this type.

Type B is represented by a top-necrosis virus that is carried by some strains of the Dutch varieties Monocraat and Roode Star and is distinct from the viruses of type A, since it produces necrotic symptoms in Duke of York, a carrier for the viruses of type A, and in Zeeland Blue, a variety in which the viruses of type A are semilatent and cause a mosaic.

The virus of yellow dwarf may perhaps be included in this section. According to the description of Barrus and Chupp (5), this disease resembles top necrosis. From a histological point of view their description, however, is very incomplete. It differs from the viruses of types A and B in that the symptoms it produces appear in inoculated plants of the variety Green Mountain when grown at high summer temperatures, but fail to develop in this variety when the inoculated plants are grown in the cool summer climate of Holland. Fernow, in his letter acccompanying the tubers sent from America, wrote that the development of this disease is favored by growing the plants at 15° C., until they are 5 to 6 in. high and then transferring them to a temperature of 25° C.

That Hoggan did not find x-bodies in healthy American Green Mountain does not seem to exclude the possibility of their occurrence in varieties showing symptoms of top necrosis (*cf.* 6).

SECTION IV: ACROPETAL NECROSIS

Necrosis chiefly in the collenchyma of the leaf veins, petioles, and stems, in cases extending gradually to other tissues, no restriction by a cork cambium. The advance along the stem is acropetal. Dropping of lower leaves. Rugose mosaic (Schultz and Folsom) and a part of the diseases collectively known as crinkle and streak are characterized by this type of necrosis.

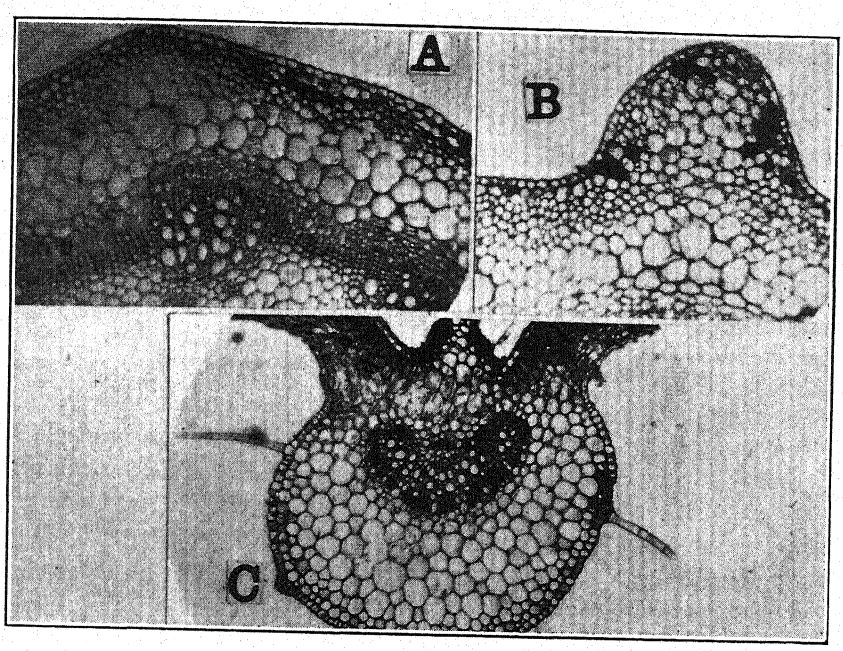


FIG. 3. A. Necrosis beginning in the collenchyma of the stalks as it occurs in some Dutch and American potato varieties that have been grafted with scions of apparently healthy plants of the old Dutch varieties Zeeland Blue, Botergele, Bloemgraafjes, and Gladblaadjes, and of the new Dutch variety Thorbecke. This necrosis is the histological characteristic of the disease described as acropetal necrosis in this paper. Some related but not identical forms of this disease occur in Europe and America. They have been described as streak, stipple streak, crinkle, and rugose mosaic. B. The same "collenchyma necrosis" in the "wing" of the stalk. C. The same necrosis as that found in the collenchyma adjacent to the veins of the most strongly downward-curved parts of the leaves of plants suffering from crinkle or rugose mosaic. The strongest necrosis is visible in the collenchyma adjacent to the upper side of the vein; the necrosis also is visible in two spots of the collenchyma adjacent to the under side of the vein.

In most of the types described as crinkle and in rugose mosaic, the same necrosis of the collenchyma is found as is typical for acropetal necrosis, but it is largely restricted to the collenchyma adjacent to veins of those parts of the leaves that are most strongly curved downward (Fig. 3, C);

the necrosis does not extend to the petioles and stems, nor do the tubers of the plants suffering from rugose mosaic or crinkle show distinct effects of the disease. The perpetuation symptoms in crinkle may be somewhat more severe than the inoculation symptoms, but they are of the same type and are annually recurrent. Yet, rugose mosaic, crinkle, and streak are very closely related; in both, the lower leaves, in which the collenchyma necrosis is most developed, drop prematurely, and the same virus in one variety may produce a rugose mosaic or crinkle and in another variety a streak.

The virus causing rugose mosaic has a rather high thermal death point (41).

No masking of symptoms by high temperatures occurs in this group. No x-bodies have been found (34).

Quanjer and Oortwijn Botjes (69) have described different types of this group occurring in Holland. In type A, Atanasoff's acropetal necrosis, necrotic spots adjacent to the veins and consequently angular appear in the lower and middle leaves, not on the top leaves (Figs. 4, B; 5, B; and 6, B), the necrosis beginning in the collenchyma of the leaf veins, leaf stalks, and stems (Fig. 3, A and B); superficial necrotic areas appear also on the tubers (Fig. 7, B).

The second-year plants as a rule are small, show severe crinkling, and are brittle as glass; their leaves are spotted, their veins are brown, their lower leaves drop off early in the growing period, and their stems are heavily striped. The necrosis extends to the interior and the plants die prematurely; the tubers are small and the necrosis extends deeper in them than in the first-year tubers. They die, as a rule, during storage.

This disease shows complete symptoms in the varieties Paul Krüger, Duke of York, and Green Mountain; it occurs in a semilatent form resembling Murphy's crinkle or Schultz and Folsom's rugose mosaic in the varieties Bravo and Rose 4 and in a still more suppressed form as mild rugose mosaic in the variety Eigenheimer. It is present in an entirely masked form in the old Dutch varieties Zeeland Blue, Botergele, Bloemgraafjes, and Gladblaadjes. A somewhat more virulent type of this disease occurs in a wholly masked form in the Dutch variety Thorbecke.

Attenuation of the streak virus carried by Zeeland Blue has been brought about by the writer by allowing it to pass through a potato seedling which itself acted as carrier. For this reason differences in severity of the symptoms are not to be regarded as a reason for classifying a virus as a different type.

A rugose mosaic in Cowhorn from America behaves in Paul Krüger as an acropetal necrosis, which is not distinguishable from that of type A.

What seems to be another type (B) of acropetal necrosis has been found by Oortwijn Botjes in the fields in the variety Noordeling. In plants of

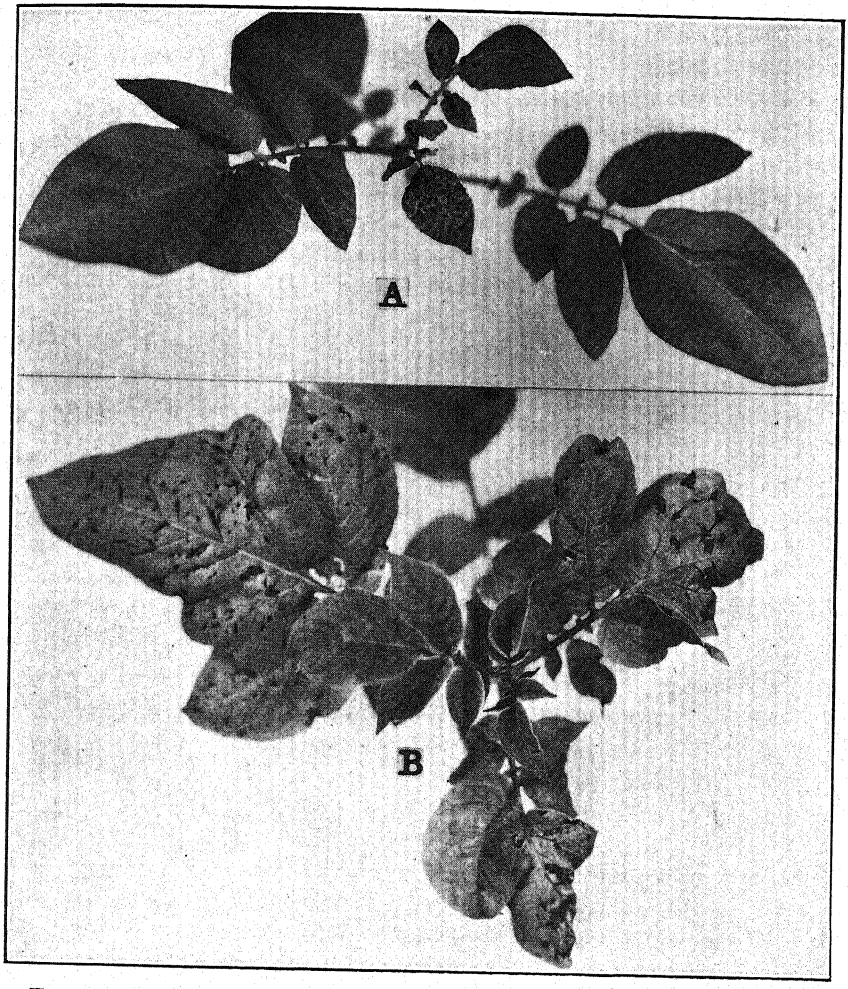


FIG. 4. A. Top necrosis or acronecrosis showing up in the tender tips of the lateral shoots of Paul Krüger (President) the stalks of which have been grafted with apparently healthy plants of Green Mountain. The necrotic spots developed within about 12 days after the grafting took place. This disease is characterized by necrosis radiating from the internal phloem in the surrounding parenchyma (Fig. 2 A. and B.). B. Acropetal necrosis showing up in the expanded leaves of Paul Krüger, which have been grafted with apparently healthy plants of Zeeland Blue. The necrotic spots developed about 12 days after the grafting took place. They extend from the collenchyma adjacent to leaf veins, petioles, and stems.

Bravo, bearing grafts of the disease occurring in the field, a type of crinkle appears somewhat different from that produced by grafting Bravo with scions of Zeeland Blue. The rugosity in the latter type of crinkle is more

severe than in the former; also, there is more mottling in the latter than in the former. When scions of Zeeland Blue are grafted on Noordeling the resulting symptoms include more leaf drop than is typical of the diseased Noordeling in the field. Each of these two viroses, which have many points in common, retains its individuality unchanged through a long term of years.

Also considered to belong to this group is rugose mosaic in Green Mountain. A difficulty in diagnosing it on the differential host Paul Krüger arises through the appearance of top necrosis in the latter. The transmission on Duke of York, which itself is a carrier of top necrosis, resulted in

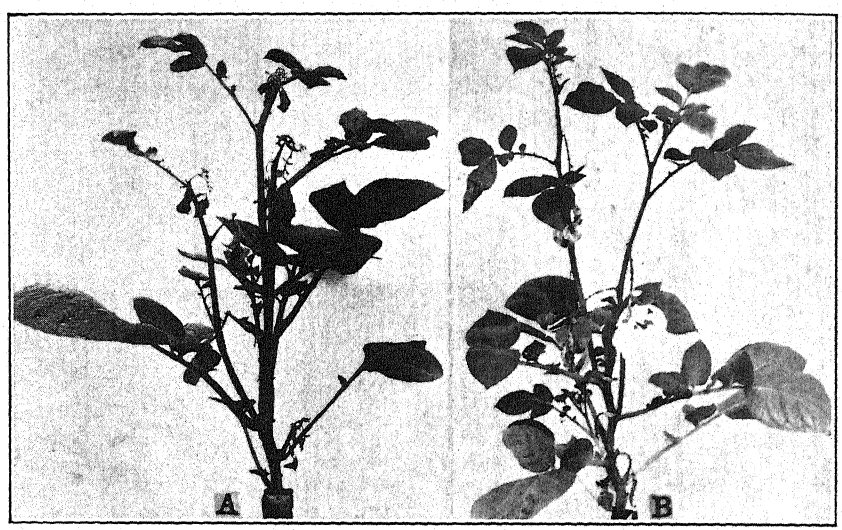


FIG. 5. A. Top necrosis or acronecrosis as seen in the differential host variety Paul Krüger 18 days after it has been grafted with the carrier Green Mountain. The tops of the lateral shoots, developing beneath the graft, are killed. B. Acropetal necrosis as seen in the differential host variety Paul Krüger 18 days after it has been grafted onto the carrier Zeeland Blue. The expanded leaves of the side shoots developing under the graft are covered with necrotic spots beginning at the collenchyma of the leaf veins, petioles, and stems. These leaves are killed and remain hanging down from their point of attachment.

the appearance of rugose mosaic in this variety. Also a crinkle in the variety Up to Date, from Ireland, behaved as rugose mosaic in Paul Krüger.

This section, no doubt, will be found to contain still further types and a classification in A, B, etc., must be looked upon as provisional in character. How far Salaman's crinkle A, para-crinkle, and streak A are related to the types thus far studied must be determined by comparison on standard varieties.

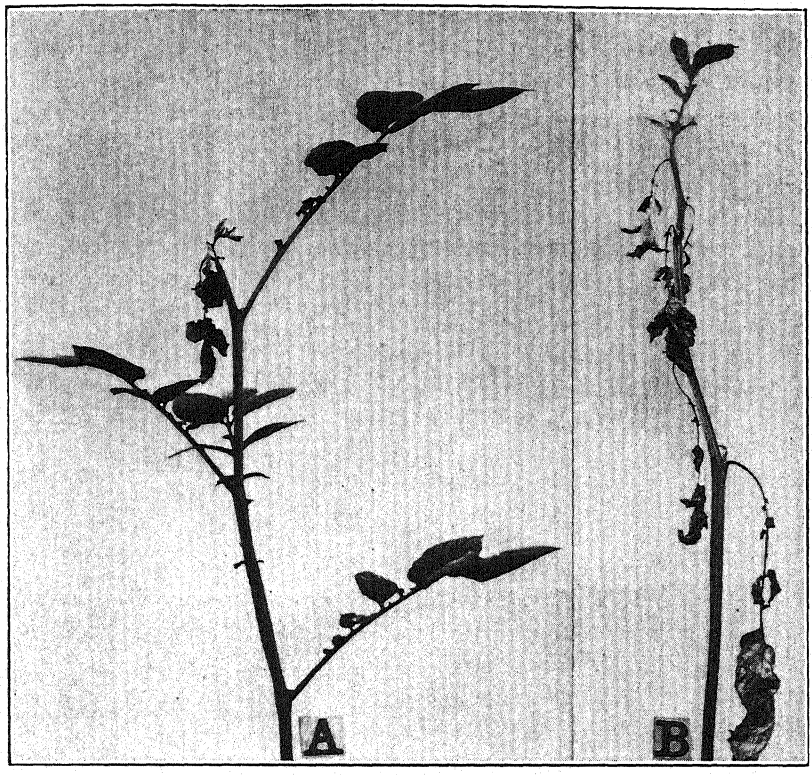


FIG. 6. A. A lateral shoot of a Paul Krüger plant 24 days after the main stalk has been grafted with Green Mountain. The top necrosis has killed the tender tip as well as a nearly developed leaf. It extended to the lower leaflets of a fully expanded leaf; necrotic spots are developing on the other leaflets of this leaf. The lower leaves remain free from spotting. B. A side shoot of a Paul Krüger plant 24 days after the main stalk has been grafted with Zeeland Blue. The leaves of this side shoot were covered with angular necrotic spots beginning at the collenchyma of the ribs and extending over the petioles and the stem. The youngest leaves at the top remain free from symptoms, as is typical for acropetal necrosis.

Whereas the types A and B of top necrosis described above differ from each other in the range of varieties that show symptoms and are, in this respect, comparable to the biotypes of the rust fungi, the types of acropetal necrosis mentioned here show, besides differences in the range of reacting hosts, also slight differences in the type of symptoms produced and for this reason are comparable to the biotypes of *Colletotrichum Lindemuthianum*.

It would be extremely interesting to know if the relation of the members of the acropetal-necrosis section also can be proved by Johnson's methods.

SECTION V: PHLOEM PARENCHYMA NECROSIS OF THE TUBER (PSEUDO-NETNECROSIS)

Necrotic spots in the storage parenchyma next to the external and internal phloem of the tubers only; no foliage symptoms. The disease is perpetuated by the seed tubers. The necrotic areas are spot-like rather than blotchy (as in top necrosis). The disease does not resemble netnecrosis (*cf.* Fig. 8, A and B) with which it has been confused by Atanasoff (4), hence the name pseudo-netnecrosis, which the writer originally gave to the disease, but which has the disadvantage of being derived partly from English and partly from Greek. Therefore, the name phloem-parenchyma necrosis of the tuber may have advantages for international use. Phloem-parenchyma necrosis of the tuber is sap- and aphis-transmissible (68). High temperature during tuber storage encourages the development of the symptoms.

A disease called "vererbliche Eisenfleckigkeit" by Fruwirth (25) is probably caused by the same virus.

SECTION VI: CONCENTRIC NECROSIS OF THE TUBER

Necrotic spots in the storage parenchyma demonstrating themselves on the cut surface as concentric brown rings arising from some point on the skin, often a lenticel. This disease is not perpetuated by the seed tubers. The rings (Fig. 8, c) are composed of necrotic cell groups which resemble in their microscopical features those inside and outside the xylem in phloem-parenchyma necrosis (42). The cell groups, however, are arranged in concentric rings in concentric necrosis. Infection takes place from the soil, evidently through some of the lenticels (*cf.* Chapter 1). There is no transmission by seed tubers, (92), (4), (67).

The disease is already developed at the end of the growing period, hence the wound-cork tissue around the necrotic spots is more extended than in phloem-parenchyma necrosis.

The disease has the popular name "kringerigheid" in Holland. It is known in Germany under the name "Pfropfenbildung" and a part of the German "Eisenfleckkeit" and the American and English "internal brown spot" or "internal rust spot" may be identical with it. In the French paper of Schwellengrebel the disease is called "Maladie des tâches en couronne."

DISCUSSION

One can hardly deny the existence of a whole world of organisms extremely small, invisible or hardly visible, and not yet cultivable apart from the living host, that cause plant viroses. The multiplication of all the viruses in their hosts, the occurrence of what seem to be species, varieties,

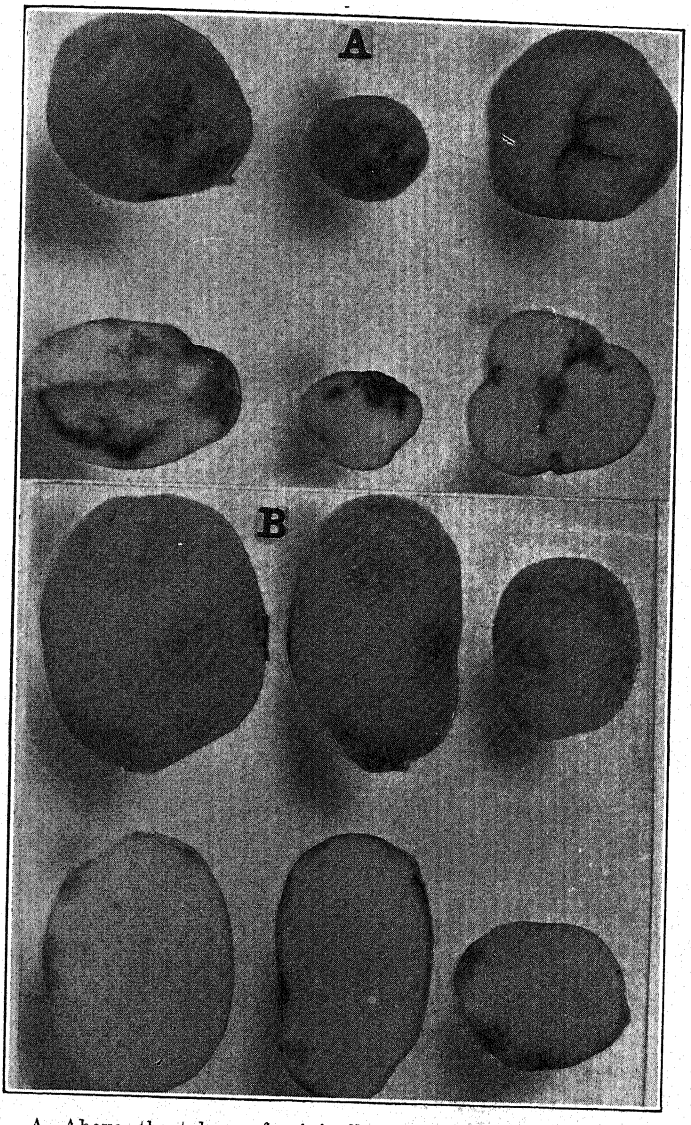


FIG. 7. A. Above, the tubers of originally virus-free plants of the varieties Paul Krüger, Bevelander, and Kerr's Pink, dug 3 to 4 months after the mother tubers had been core-grafted with an apparently healthy Green Mountain carrying top necrosis or acronecrosis. Below, the same tubers, cut. The necrosis radiates from the internal phloem and may kill the apical eye. B. Above, the tubes of originally virus-free plants of the varieties Paul Krüger, Duke of York, and Green Mountain, dug 3 to 4 months after the mother tubers had been core-grafted with apparently healthy Zeeland Blue carrying acropetal necrosis. Below, the same tubers cut. The necrosis is superficial, agreeing with what can be seen in the stalks (where it begins in the collenchyma).

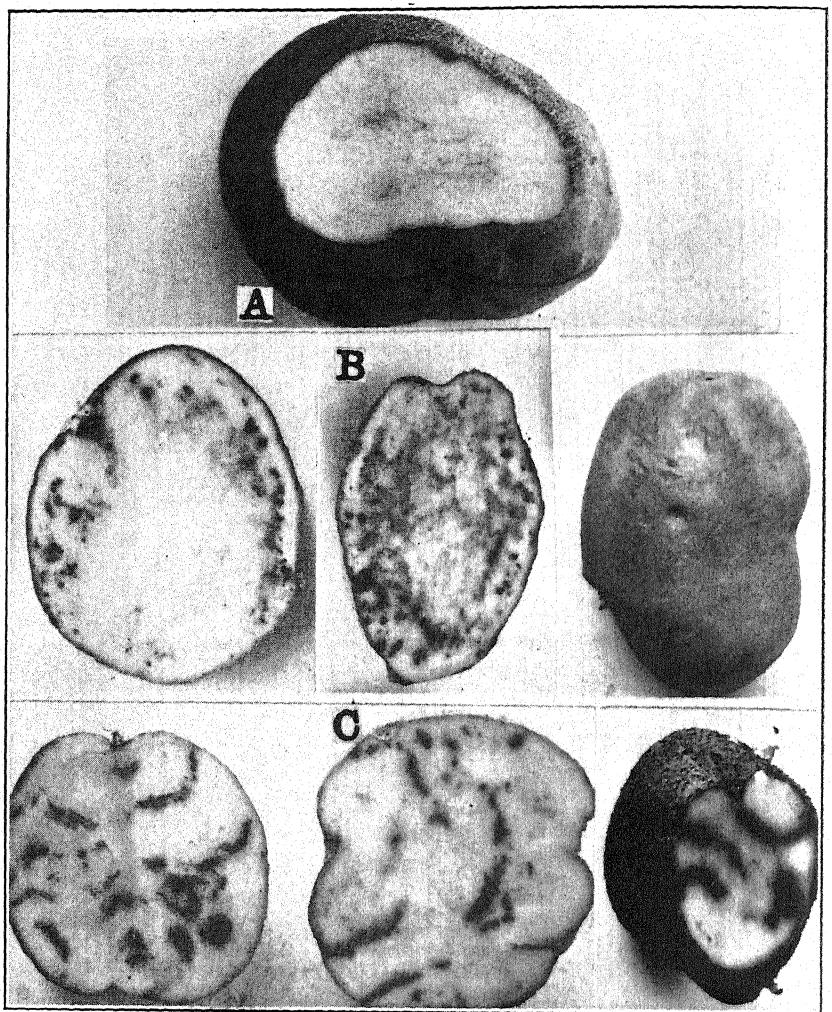


FIG. 8. A. Netnecrosis (phloem necrosis in the tubers of Green Mountain) for comparison with B and C. B. Pseudo netnecrosis, or phloem parenchyma necrosis of the tuber, at the left and in the middle in variety Roode Star, the necrotic spots are in the phloem parenchyma inside and outside the xylem ring. At the right in variety Paul Krüger, where the necrotic spots are visible at the surface near the heel end. C. Concentric necrosis of the tuber at the left in the Dutch varieties Alpha and Roodeschoolsche Jam, at the right in the German variety Direktor Johansen, where it is visible at the surface.

and biotypes, the phenomena of attenuation, and increase of virulence suggest their parasitic nature. Also, the "waiting period" of some of the viruses in the bodies of the transmitting insects, evidence for which has been

furnished by Severin, Kunkel, Elze, Storey, and Smith, is, in some cases at least, suggestive of parasitism. This holds true especially for the virus of aster yellows with its long incubation in *Cicadula sexnotata*. The impossibility of growing the viruses *in vitro* carries with it the impossibility of differentiating between them by means of chemically different nutrient media or by differing chemical reactions on identical media, as is done with bacteria. This is the reason why identification and differentiation are dependent largely on symptomatology.

Earlier descriptions of the morbid symptoms in plants suffering from viroses are frequently not specific, detailed, or accurate enough. For this and other reasons, Johnson presented a classification of some tobacco viroses chiefly on the basis of physical properties determined in the virus-containing juices and their behavior towards treatment by chemicals. Later he applied the same method of classification to some of the potato viroses. While such direct methods offer great possibilities, especially for the knowledge of the nature of the viruses concerned, it can not be expected from these methods that they give any information on the properties of the viruses which interest us most, *i.e.*, their properties as pathogenes.

It has here been shown that the indirect method of symptomatology can be improved by histological and physiological refinement in connection with appropriate hosts, appropriate transmission, and control of environmental conditions and that a classification and nomenclature of some potato viruses, at least, is possible on the basis of well-defined pathological conceptions. To terminate the indiscriminate use of confusing names for an ever-increasing number of mosaics, crinkles, and streaks, it is proposed to adopt the nomenclature presented in this paper and to develop it further.

Furthermore, it is suggestive for the study of the nature of the viruses concerned to observe that, at least in those varieties which are liable to necrosis, they select different plant tissues on which to exert their enzymes and toxins. The leaf-roll virus acts on the sieve tubes and companion cells; the top-necrosis viruses, on the parenchyma surrounding the internal phloem; the viruses causing acropetal necrosis, on the collenchyma first; the virus causing phloem-parenchyma necrosis, on the storage parenchyma of the tubers, only; while some of the mosaic viruses thus far seem only to injure the mesophyll of the leaf without causing actual necrosis.

There is an interesting morphophysiological problem in the correlation of a necrosis radiating from the internal phloem with the killing of the tender tips and in the correlation of a necrosis extending from the collenchyma with a killing effect advancing acropetally. The solution of this problem must be the aim of future work.

The difference in plant tissues attacked must be due to chemical differences in the causal agencies. Although classification of plants and

animals, as a rule, is based on morphology, it is in some cases not objectionable to classify them on a chemical basis as well. Johnson has done this, for his diagnostic features of the property type must be attributed to chemical characteristics of the viruses concerned.

And in a more general way we may take it as granted that even form in the vegetable and animal kingdom is a function of chemical processes. Thus, chemical agreements or differences may be of taxonomic value. This being true, the basing of a classification and a nomenclature of viruses on clearly definable necrotic effects, typical for each of them, may be considered a valid principle in virological studies.

SUMMARY AND CONCLUSION

From the discussion in the foregoing on the principal methods of identification, differentiation, and classification of plant viruses, and their respective advantages and disadvantages, we may conclude that our present knowledge is insufficient for classification of plant viruses and that the method of morbid anatomy and physiology thus far is somewhat neglected.

A standard named collection of potato-virus material from the leading workers of the Old and the New World being available, it was attempted to find a new basis for the classification and nomenclature of the viroses of the potato—based on such characteristics as can be recognized by everybody able to use a microscope.

The principle has been followed that a potato virus should be identified, named, and classified after the morbid effect it has on a variety that shows clearly definable internal symptoms. None of the Solanaceae other than the potato have been used to test the viruses for this purpose. For international corroboration and agreement on nomenclature, a stock of the same differential host varieties should be grown in a virus-free state at all research stations where potato-virus studies are in progress. One of the best differential hosts is the variety Paul Krüger, called President, in England.

To avoid further trouble resulting from the use of descriptive names for an ever-increasing number of mosaics, crinkles, streaks, and spottings, the writer proposes the following as an international nomenclature for the potato diseases of the virus type:

Anecrotic mosaics (the result of later work may be that some of the members of this section must be classed under one or more of the groups of necrosis).

Phloem necrosis (containing thus far one disease, called leaf roll, or as inoculation symptom in tubers of Green Mountain, net necrosis).

Acronecrosis (or top necrosis, a disease produced by healthy-potato virus occurring in most of the American standard varieties and in a Scotch

and a French variety. The disease shows up after grafting these carriers on to a differential host).

Acropetal necrosis (containing rugose mosaic and a part of the diseases hitherto called crinkle and streak. Viruses belonging to this group may be latent in some European varieties, which consequently behave as carriers).

Phloem-parenchyma necrosis of the tuber (*Pseudo-netnecrosis*) (no foliage symptoms belong to this disease, which is perpetuated by the seed tubers).

Concentric necrosis of the tuber (no foliage symptoms. This disease is not perpetuated by the seed tubers; infection takes place from the soil, obviously through the lenticels).

Short descriptions of the histological features characteristic for each of the six respective sections are found in the eighth chapter.

It is hoped that the grouping of potato viroses after the system outlined in this paper will, in future investigations, prove consistent with results obtained through other ways of approaching the problem, and that the system, still in a tentative stage, may appear applicable to other viroses not yet sufficiently studied histologically.

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THE INHERITANCE OF THE REACTION OF MAIZE TO GIBBERELLA SAUBINETII[1]

K. G. McIndoe

INTRODUCTION

The use of resistant varieties in the control of root-rotting diseases of maize is universally recognized as the method giving most promise. A prerequisite to the production of such varieties is the isolation of resistant selfed lines, the resistance of which is then to be combined with advantageous characters of other lines in producing the desired varieties. It is in the combination of such lines that knowledge of the manner of inheritance of reaction to the disease becomes useful. The present investigation was conducted mainly with the intention of obtaining this information.

Gibberella saubinetii (Mont.) Sacc. of late years has been recognized as largely contributory in causing root and stalk rots of maize in the Corn Belt of the United States. Investigation of the disease in Minnesota has mostly centered around this pathogene. Its deleterious effect is most obvious upon the seedling, and, according to the extent of injury, the reddish-brown lesions may be purely local on coleoptile, coleorhiza, mesocotyl, and primary and seminal rootlets, or may involve the greater part of the developing embryo. In extreme cases, the mesocotyl (the structure between the base of the coleoptile and the attachment of the scutellum) may be entirely rotted through, in which case the food supply to the young leaves is cut off and the plant wilts. Early production of adventitious roots from the first node may enable the plant to avoid destruction, even though the mesocotyl may be well rotted. Such plants may appear to be perfectly healthy above the ground and, indeed, may remain so. In others the adventitious roots do not appear early, the leaves do not receive adequate nourishment, and the plants either die or linger on in an attenuated fashion. The study of the inheritance under discussion necessitates the isolation of selfed lines of maize which exhibit decided differences in their degree of resistance. At the initiation of the investigation herein described such differences had

[1] This study is a phase of the investigations conducted by the Division of Agronomy and Plant Genetics, in cooperation with the Division of Plant Pathology and Botany, on the general problem of disease resistance in plants. A thesis presented to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author wishes to express his indebtedness to Dr. H. K. Hayes for direction and criticism throughout the study. To Dr. E. C. Stakman and to Dr. J. G. Leach his thanks are also due for criticism and suggestions regarding the manuscript. Published with the approval of the Director as Paper No. 973 of the Journal Series, Minnesota Agricultural Experiment Station.

already been broadly established by greenhouse experiments conducted by the Divisions of Agronomy and Plant Genetics and Plant Pathology and Botany, at the Minnesota State Agricultural Experiment Station. The lines thus distinguished were therefore available. In dealing with such a quantitative character as disease reaction, the experimenter is indeed fortunate if he has immune parental stock, *i.e.*, lines homozygous for complete resistance. The material available in this study was in no way remarkable in possessing this desirable attribute; in fact, as will be shown later, certain environmental conditions would transform apparent high resistance into complete susceptibility. However, under the circumstances, resort was had to the best that offered, remembering that the variability of the reaction was likely to preclude any simple explanation of the results.

PREVIOUS INVESTIGATIONS

The more strictly pathological phases of cereal root-rotting diseases are naturally fundamental to any study of the inheritance of such diseases. Symptomatology of the diseases, identification of the pathogenes involved, their relative virulence, and their distribution are aspects of the matter that seem scarcely relevant in this paper, and consequently detailed reference to such work may well be omitted. Suffice it to say that the recognition of the predominant influence of *Gibberella saubinetii* by Hoffer, Johnson, and Atanasoff (5), as well as by Dickson, Johann, and Wineland (3), in causing root rot of maize focussed attention upon its potentialities.

The study of seedling reaction of maize and wheat to *Gibberella saubinetii* hinges largely about the work of Dickson and Holbert. Dickson (1, 2) demonstrated the relation between the environment and the expression of disease symptoms. He showed that the temperature of the soil is the most important single factor determining the extent of seedling blight and that, while temperatures of 12° to 28° C. favored the blighting of wheat, maize seedlings were more subject to attack between 8° and 20° C. Resistant and susceptible strains were isolated, and, while the latter succumbed at temperatures lower than 24° C., the former were not attacked until grown at temperatures lower than 16° C. Dickson's explanation of the difference in reaction of wheat and maize involved consideration of the changes in metabolism of the host produced by differences in temperature, so that the manifestation of disease was conditioned more by host reaction than by fungus response to the varying temperatures. At temperatures unfavorable to the growth of the host, its metabolism became "unbalanced" and predisposition to attack was increased.

The same author (1) also found that the extent of blighting of wheat seedlings—other factors being favorable for infection—was directly propor-

tional to the amount of infestation, *i.e.*, to the amount of inoculum in contact with the seedling. With wheat, also, he showed that low soil moistures increased the percentage of blighted seedlings at all temperatures.

That factors other than intrinsic metabolic processes may be operative in conferring apparent resistance or susceptibility has recently been suggested by Peterson (9). While admitting the influence of such intrinsic processes, he would prefer to attribute the apparent resistance of seedlings to the power possessed by them of producing a functioning nodal root system before the mesocotyl becomes completely rotted by the fungus. The leaves of such strains as are not capable of the early production of nodal roots are wilted quickly as a result of the severance of their food supply.

It is probable that a combination of both intrinsic resistance and such "avoidance" of destruction is active in producing the final result. It becomes apparent, however, that the influences which determine the estimated degree of resistance are by no means simple and that we are dealing with a complex reaction.

A further factor that may be connected with resistance of the mature plant has been suggested by Hoffer and Carr (6). Their contention is that plants which contain the largest quantities of aluminum and iron salts appear to develop the most severe cases of root rot, provided other conditions are favorable for infection. Deficiency of available phosphates also seems to favor fungous infection. They suggest that the power of selective absorption of these metals may be hereditary and thus influence the incidence of disease.

Dickson and Holbert (4), in preliminary studies on the inheritance of disease reaction, come to the only conclusion appearing in the literature on this subject. They state that "first-generation hybrids between (these) resistant and susceptible strains are susceptible to seedling blight at all temperatures. That is, susceptibility is dominant in such crosses." They give no experimental details in support of this statement.

EXPERIMENTAL MATERIALS

The parental cultures employed in the inheritance investigations consisted of 27 selfed lines of maize, comprising 3 varieties, viz, 9 lines of Minnesota 13, 10 lines of Rustler, and 8 of Northwestern Dent. All these lines had been selfed previously from 7 to 14 years and therefore could be assumed to be reasonably homozygous. Within the lines of each variety, all possible crosses had been made in 1927, 1928, and 1929, so that, with the exception of a very few crosses unavoidably lost, almost all these F_1 crosses were available for testing in the greenhouse. Wherever possible, the most recently produced seed was used, but, if not available, recourse was had to

seed from the previous year. A total of 105 F_1 crosses were thus employed. Preliminary tests were conducted in the winter of 1928–29 on parental lines and F_1 crosses, but, on account of variable greenhouse temperatures, the results were not considered sufficiently reliable. However, these experiments adequately indicated broad differences between the various lines, and, with this knowledge, certain crosses were selected for further study in the F_3 generation.

In the winter of 1929–30, therefore, there were available the parental lines, the F_1 crosses, and certain F_3 lines obtained by growing the F_2 in the field in 1929 and self-pollinating plants at random. The crosses from which these F_3 lines originated were selected on the basis of the reaction of the parents giving rise to them. Five such crosses were selected as follows:

Minn. 13	43 × 46	Resistant × Susceptible
Minn. 13	43 × 47	Resistant × Susceptible
Minn. 13	49 × 50	Susceptible × Intermediate
Rustler	58 × 60	Susceptible × Susceptible
N. W. Dent	64 × 66	Intermediate × Susceptible

It was somewhat unfortunate that of all the lines tested only one, 43, could be called resistant. Consequently a cross Resistant × Resistant could not be selected.

Throughout the study, a single physiologic form of *Gibberella saubinetii* was used, which originally was obtained by the Department of Plant Pathology from L. R. Jones of Wisconsin and designated by Tu (10) as *Fusarium graminearum* form I. Of the three forms identified by Tu, this form was found by him to be most virulent in causing head blight of wheat, and preliminary experiments conducted in connection with the present investigation indicated that its pathogenic properties were similarly manifested in producing root rot of maize. It was also distinguished by its ability to form perithecia in pure culture, a property not displayed by the other two forms.

EXPERIMENTAL METHODS

As has already been indicated in the introductory section, environmental conditions modify considerably the expression of disease symptoms. Consequently, reduction of variability in such conditions becomes an essential if reliable bases of comparison are to be made. Temperature, as well as soil moisture, light intensity, and amount of inoculum are the chief factors to be considered, and, of these, variation in temperature creates the greatest disturbance.

Absolute control of temperature in a large greenhouse is of course quite impossible; nevertheless, by the use of radiators equipped with blowers

automatically regulated by thermostats, it was found that the air temperature could be controlled during the winter months within the limits of about 3° C.

By maintaining the air temperature as near as possible to 20° C., the soil temperature as a rule approximated 15° C. This temperature was sought, for, though low enough to permit manifestation of infection in the great majority of lines, it still allowed sufficient growth of the plants. With the variation in air temperature, the soil temperature naturally fluctuated concomitantly, though never to the same extent, owing to lag. As a general rule, the variation in soil temperature was rarely more than 2° C. about the selected temperature (15° C.). A diurnal fluctuation was always present, with the highest temperatures from noon to 4 p. m. and the lowest from 4 a. m. to 8 a. m. This fluctuation was particularly accentuated if the sun happened to shine during the day. The air and soil temperatures were recorded on a thermograph actuated by two thermocouples, one buried 2 inches in the sand and the other supported about 4 inches above the surface. Owing to the fact that the radiator in each greenhouse was situated at one end of the house, it was necessary to maintain air circulation by judicious placing of electric fans about the floor. Even with this precaution, it was frequently found that an absolutely uniform distribution of temperature was difficult of attainment, so that local regions of the sand benches tended to show divergences of temperature of about 2°. This divergence may seem small, but its effect as expressed by the survival of the plants was plainly noticeable.

The seedlings were grown in clean, freshly dug sand of a fairly coarse texture, which could be considered reasonably free from pathogenic organisms likely to vitiate results. This sand was watered every day and thus kept quite moist. Two greenhouses were used, and each contained two benches running longitudinally at waist height, and about 6 inches in depth. One bench in each house was used habitually for inoculated seeds and the other planted with seeds of similar strains treated with a Semesan solution. Limitation of space, as well as of time, naturally puts a definite restriction upon the extent of trials designed for the purpose of reliable appraisal of relative resistance or susceptibility. The method finally adopted was to plant 60 seeds of each parental culture, F_1 cross, or F_3 line. These seeds were distributed as follows: Thirty were inoculated with the pathogene and planted immediately in two replicates of 15 seeds each. The remaining 30 were treated with the Semesan solution and also planted in two replicates of 15 seeds each, thus serving as checks. Earlier experiment had shown that very little difference was discernible between the plants from seed planted without any treatment whatsoever and plants from those treated with Semesan. Consequently, it was concluded that

extraneous organisms already located upon the seed or possibly in the sand had little effect in influencing the incidence of disease symptoms on seedlings. Nevertheless, in order to obtain an accurate estimate of the germination and growth potentialities of the lines tested, the check plants were always treated with Semesan before planting.

To expedite the inoculation and planting of the seeds, the following procedure was adopted: The sand in the benches was thoroughly wetted, and holes each 1½ inches deep were stamped in it for the reception of the seeds. For this purpose, a stout board studded with pegs arranged in rows 2 inches apart and an inch between the pegs was employed. Hence the various lines could be planted at a uniform depth and in a regular successive manner. In inoculating, each group of 15 seeds was immersed for a few seconds in a solution containing a mixture of spores and mycelial fragments and immediately planted. The sand was then lightly raked over, so as to cover the holes. The inoculum was prepared by mixing tap water with a pure culture of *Gibberella saubinetii* which had been growing for about 10 days on a moist mixture of wheat and oat grains. The mixture was strained through cheesecloth to remove the grains. Throughout the experiments, an effort was made to maintain the concentration of the inoculum as uniform as possible by so diluting it until about 30–50 spores were visible under the low-power field of the microscope. Planting and treatment of the check plants were carried out in an exactly similar manner, except that a 0.02 per cent solution of Semesan was substituted for the spore suspension.

About 4 weeks after planting, the seedlings reached a stage suitable for note taking. To illustrate the method adopted in this work, an excerpt from the notebook is given below:

Culture number	Greenhouse number	Total number of seedlings	Number stunted	Number not appearing	Vigor	Number free from infection	Number locally infected	Number completely infected
3	A 8	3	2	12	1	.....	.....	15
	B 8	5	2	10	1	.....	.....	15
	C 8	12	.....	3	3			
	D 8	9	.....	6	3			
4	A 9	13	1	2	4	7	2	6
	B 9	12	3	3	3	2	2	11
	C 9	15	.....	.....	5			
	D 9	15	.....	.....	4			

The letters A and B represent the replicates inoculated with Gibberella, while the letters C and D represent these replicates treated with Semesan.

In any line, some plants generally appear, above the ground, to be quite healthy and they remain so. Others reach a height of ½ inch to 2 inches. and then, because of destruction of the subterranean parts by the fungus, begin to wilt and finally become stunted or die. Still others do not appear at all. Therefore, provision is made in the notes for these characters. In obtaining the notes on vigor, an arbitrary scale was set up ranging from 1 to 5. Thus, a line possessing highest vigor, as judged by its general aspect, was designated as 5, one possessing medium vigor as 3, one showing little vigor as 1, and so on. Skill in the allocation of indicative numbers is a matter of experience, which a little practice soon gives.

In estimating the degree of resistance possessed by each line when grown under conditions favoring infection, some criterion becomes necessary. Although an attempt is made to maintain the seedlings of any culture under exactly similar environmental conditions, it is obvious either that such uniformity rarely obtains or, else, the seeds in each selfed line or F_1 cross are not homozygous, for some seedlings remain healthy, while others in the same culture may wilt or die even before emergence. (Figs. 1, 2, 3, and 4). We presuppose that homozygosity exists, so that we must conclude that a state of "balance" exists between the forces contending for the life of each culture. A few cultures are able to withstand wholly the inroads of the fungus; in some slightly varying opportunities for infection lead to the death of some seedlings and the continuance of others. It is therefore reasonable to assume that the higher the degree of resistance, the greater the number of surviving plants in each culture. Consequently, the number of plants appearing healthy above ground was employed as a basis for estimating the degree of resistance. To arrive at this number, the "number stunted" was subtracted from the "total number," in each case. Thus was obtained a number for each replicate, varying from 0 to 15. But it was considered that this number was subject to some correction, due to its being influenced to some extent by the germination capability of each culture. Accordingly, a correction was made on the basis of the germination percentage of the same culture in the Semesan-treated replicates. Thus, for instance, if in these two replicates a total of 25 seedlings emerged out of a possible 30, the number of healthy plants in each of the inoculated replicates was multiplied by 30/25, and the resulting figure used as the final index of resistance. This correction for germination was applied throughout the whole study. If the number allocated to a culture approached 30, then the line was considered resistant; if nearer zero, the line was considered susceptible. A complete range extended between these two extremes.

Admittedly, this scheme is by no means wholly accurate or indeed reliable in making fine distinctions between lines of nearly corresponding

resistance. The number of plants appearing healthy is the resultant not only of genetic potentiality but also of influences such as temperature, moisture, germination capability, and differences in inoculation. These factors all tend to obscure the intrinsic powers of resistance of the various lines, and their easily variable nature is capable of radically influencing the final result.

The notes in the three last columns in the notebook were taken with the intention of finding out to what extent lesions on the subterranean parts influenced the appearance of the subaerial parts. The headings of the columns are self-explanatory. To obtain this information, every plant was dug up, after earlier notes had been taken, and macroscopically inspected to ascertain the extent of the lesions.

EXPERIMENTAL RESULTS

The reliability of the method of experiment as a means of ascertaining inherent resistance to Gibberella saubinetii: Limitation of material compels a compromise between expedition of the experiment and more extensive and perhaps more accurate investigation. As stated in the foregoing section, the method adopted was to grow 15 seeds of each culture in duplicate, in both the inoculated and the check material. In figure 1 the difference between the inoculated and Semesan-treated plots can be seen readily. It will be noted that in each of the two crosses illustrated by figure 1 the F_1 exhibits some degree of resistance, while, of the parents of each cross, only one, viz, culture 43, shows a high degree of resistance. As a means of evaluating the degree of constancy of reaction, the correlation between the two replicates of the inoculated cultures was ascertained. The values correlated were those obtained after the correction had been made for germination percentage. The correlation coefficients were calculated for the parental lines, the F_1 crosses, and for the F_3 lines originating from five different crosses. They are given in table 1.

It will be observed that the correlation coefficient in the case of the selfed lines is negative, is low for the F_1 crosses, and considerably higher in the case of the F_3 lines originating from the crosses 49×50 and 58×60, while in the other three examples 43×46, 43×47, and 64×66, the coefficient is high and significant. There are several reasons for this variance. The F_1 crosses were tested in the greenhouse at a different and earlier period than the F_3 lines, and the average temperature of the soil over this period was about 1° to 2° C. below the average temperature at which the F_3 lines were tested. This was due to faulty placing of the thermocouples, which registered the temperature at a spot constantly higher than the greater part of the sand in the benches. The result was that the number of surviving plants in each replicate was reduced materially. Conse-

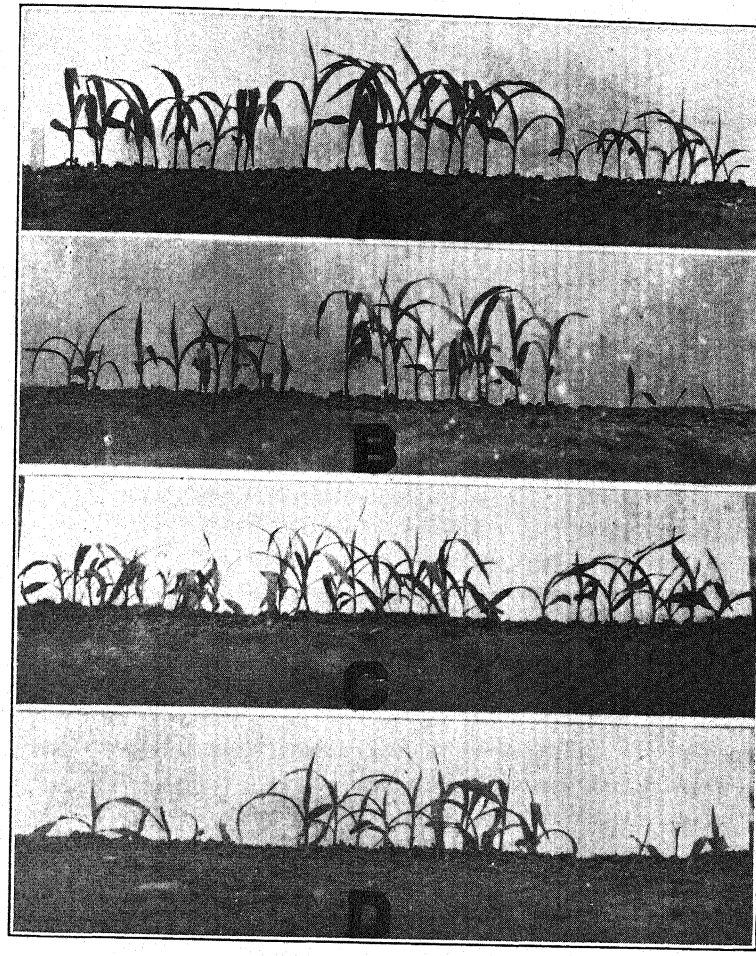


FIG. 1, A. Check plots from Semesan-treated seed under controlled temperatures in the greenhouse. Left, culture 43; right, culture 46; center, culture 43 × culture 46. B. Inoculated plots, seed immersed in an emulsion of spores and mycelium of *Gibberella saubinetii*. From left to right, same cultures as in A. C. Check plot; left, culture 49; right, culture 50; center, culture 49 × culture 50 (compare with Fig. 4). D. Inoculated plot; left, culture 49; right, culture 50; center, culture 49 × culture 50.

quently, the range of values obtained from the selfed lines and F_1 crosses was narrowed considerably in comparison with that from the F_3 lines. This naturally would have the effect of reducing the magnitude of the correlation. In addition the number of plants in each plot (15) is already low enough for a reliable test, and when this number is reduced to 2, 3, 4, or 5, small differences between corresponding plots have a disproportionate effect upon the correlative value. Moreover, on account of the lower tem-

TABLE 1.—*Correlation coefficients between the first and second replicates of selfed lines, F_1 crosses, and F_3 lines when grown from inoculated seed. The letters R, I, S represent "resistant," "intermediate," and "susceptible"*

	Number of individuals in each population	Type of cross	Correlation coefficient
Selfed lines	27	.....	$-.034 \pm .130$
F_1 crosses	100	.....	$.061 \pm .067$
F_3 lines from 43×46	60	R × S	$.608 \pm .055$
F_3 lines from 43×47	90	R × S	$.493 \pm .054$
F_3 lines from 49×50	45	S × I	$.260 \pm .093$
F_3 lines from 58×60	70	S × S	$.203 \pm .077$
F_3 lines from 64×66	31	I × S	$.599 \pm .077$

perature, a few plots failed to show any plants at all, while perhaps 3 or possibly more appeared in their respective duplicates. When the correction for germination was applied the divergence between such plots was further increased, for only one indicative number was affected. In the F_3 lines the average temperature (computed by taking the mean of the temperatures at 4-hour intervals over a period of the first 20 days) was about 15° C. The result was that the survival number in each plot was on the whole higher than in the selfed lines and F_1 crosses, and variations of a few plants did not affect the correlation so strongly. The correlations between the two replicates of the F_3 lines originating from the crosses 49×50 and 58×60 are lower because here, again, the numbers were reduced to some extent on account of the greater susceptibility of these lines in general. It is to be expected also that the highest correlations would be obtained from lines originating from such crosses as 43×46 and 43×47, for here the two parents exhibit a wide difference in reaction, and the range of types obtained from them would be greater.

Another contributory factor towards reducing the correlation between the replicates of all the cultures is the variation in temperature between different localized areas of the greenhouse. This was particularly noticeable when the selfed lines and F_1 crosses were tested, and a special effort was made to reduce it in subsequent trials, with some degree of success.

These results serve to emphasize the necessity in critical investigation of maintaining not only a suitable but a uniformly distributed temperature in the greenhouse over the period of trial. Increase in number of replicates, in so far as this is compatible with space, would also be conducive to greater accuracy. It may be said, then, that with such improvements, the method of experimentation could be considered sufficiently reliable in future investigation.

The relation between the reaction of parents and that of F_1 crosses between them: In order that environmental conditions under which parental lines and F_1 crosses were grown might be as similar as possible, both parents and crosses were grown in duplicate at the same time and in the same greenhouse. According to the method previously described each line was allotted a number indicating its reaction. Thus the numbers appearing healthy in each of the two plats were corrected on the basis of the germination in the Semesan-treated material, and the two numbers so obtained added together to give the final index.

Realizing that this index can be greatly influenced by small divergences in temperature, it was considered advisable not to classify the individual

TABLE 2.—*Reaction of selfed parents and of F_1 crosses to Gibberella saubinetii. The reaction of each culture is represented by an index number. These numbers range from 0–30 and increase with apparent resistance. Each index is given as the nearest whole number to that actually calculated.*

Cross	Reaction of parents	Reaction of F_1	Cross	Reaction of Parents	Reaction of F_1	Cross	Reaction of parents	Reaction of F_1
41 × 42	5 and 6	6	46 × 47	0 and 0	16	55 × 56	5 and 0	14
41 × 43	5 " 11	12	46 × 48	0 " 9	7	55 × 57	5 " 6	10
41 × 44	5 " 3	9	46 × 49	0 " 2	11	55 × 58	5 " 0	10
41 × 45	5 " 9	9	46 × 50	0 " 5	1	55 × 60	5 " 3	4
41 × 46	5 " 0	2	47 × 48	0 " 9	9	56 × 57	0 " 6	8
41 × 47	5 " 0	14	47 × 49	0 " 2	2	56 × 58	0 " 0	5
41 × 48	5 " 9	10	47 × 50	0 " 5	12	56 × 60	0 " 3	1
41 × 49	5 " 2	10	48 × 49	9 " 2	11	57 × 58	6 " 0	1
41 × 50	5 " 5	11	48 × 50	9 " 5	5	57 × 60	6 " 3	1
42 × 43	6 " 11	15	49 × 50	2 " 5	12	58 × 60	0 " 3	2
42 × 44	6 " 3	2	51 × 52	7 " 5	7	61 × 62	0 " 6	7
42 × 45	6 " 9	13	51 × 53	7 " 9	21	61 × 63	0 " 2	9
42 × 46	6 " 0	14	51 × 54	7 " 4	11	61 × 65	0 " 4	2
42 × 47	6 " 0	5	51 × 55	7 " 5	10	61 × 66	0 " 0	12
42 × 48	6 " 9	1	51 × 56	7 " 0	12	61 × 68	0 " 4	0
42 × 49	6 " 2	4	51 × 57	7 " 6	12	62 × 63	6 " 2	16
42 × 50	6 " 5	9	51 × 58	7 " 0	10	62 × 64	6 " 6	4
43 × 44	11 " 3	25	51 × 60	7 " 3	30	62 × 65	6 " 4	11
43 × 45	11 " 9	7	52 × 53	5 " 9	13	62 × 66	6 " 0	8
43 × 46	11 " 0	8	52 × 54	5 " 4	23	62 × 67	6 " 1	14
43 × 47	11 " 0	5	52 × 55	5 " 5	19	62 × 68	6 " 4	12
43 × 48	11 " 9	9	52 × 56	5 " 0	12	63 × 64	2 " 6	20
43 × 49	11 " 2	19	52 × 57	5 " 6	16	63 × 65	2 " 4	10
43 × 50	11 " 5	12	52 × 58	5 " 0	5	63 × 66	2 " 0	4
44 × 45	3 " 9	10	52 × 60	5 " 3	12	63 × 68	2 " 4	13
44 × 46	3 " 0	4	53 × 54	9 " 4	18	64 × 65	6 " 4	16
44 × 47	3 " 0	6	53 × 55	9 " 5	13	64 × 66	6 " 0	9
44 × 48	3 " 9	5	53 × 56	9 " 0	18	64 × 67	6 " 1	0
44 × 49	3 " 2	13	53 × 57	9 " 6	10	64 × 68	6 " 4	4
44 × 50	3 " 5	15	53 × 58	9 " 0	14	65 × 66	4 " 0	2
45 × 46	9 " 0	2	53 × 60	9 " 3	15	65 × 67	4 " 1	9
45 × 47	9 " 0	10	54 × 55	4 " 5	9	65 × 68	4 " 4	24
45 × 48	9 " 9	5	54 × 56	4 " 0	15	66 × 67	0 " 1	14
45 × 49	9 " 2	5	54 × 57	4 " 6	15	66 × 68	0 " 4	21
45 × 50	9 " 5	8	54 × 60	4 " 3	14	67 × 68	1 " 4	8

lines as resistant or susceptible but to present the results directly. This is done in table 2. The corrected indices are listed as the nearest whole number to those actually computed. It will be seen that the indices of the selfed parents never approach the magnitude of some of the crosses. This in itself indicates the quantitative rather than the qualitative character of the inheritance and suggests that the genetic factors involved are multiple. The appearance of a cross showing a high index from two parents of low indices further substantiates this hypothesis, and such cases may indicate a transgressive inheritance.

It was of interest to calculate the correlations between reaction of the low-index parents and the crosses, between the high-index parents and the crosses, and between the average of the parents and the crosses. These correlation coefficients are as follows:

Between low-index parents and crosses	$.191 \pm .063$
Between high-index parents and crosses	$.114 \pm .065$
Between average index of parents and crosses	$.110 \pm .065$

These coefficients are too low to allow any definite conclusions being drawn from them, but it would seem that in this trial the low-index parent was somewhat more strongly correlated with the reaction of the F_1 cross than was the high-index parent. The fact that most of the parental lines are not remarkable for their resistance might account for this. The low correlation between the average index of the parents and that of the crosses is also scant evidence that a transgressive inheritance is operative over the crosses as a whole. The little understood "incompatibility" between certain selfed lines on crossing may possibly reduce the correlation. It is not suggested that there is a cause and effect relation between vigor and resistance, but it is possible that when two parents, themselves possessing little intrinsic resistance, unite to give a vigorous hybrid, this hybrid may be able to produce adventitious roots at an early date and thus "avoid" extinction. It is certain, however, that all cultures with well-developed adventitious roots showed a high index, though the converse was not always true, e.g., in culture 53, variety Rustler, and most crosses involving it. It might be possible to separate partially the effects of intrinsic resistance and early development of roots by using such cultures in future experiment. Cultures with strong adventitious roots sometimes exhibited lesions of considerable extent upon the mesocotyl and other embryonic structures, and sometimes they did not. The subterranean parts of all plants inoculated with the fungus were examined (about 12,000 plants in all), and in no case was any culture found free of lesions in all its constituent plants; even the most resistant culture showed only half of its plants entirely free from visible symptoms. While many lines seemingly succumbed easily to

the invasion of the parasite, some few appeared to offer resistance to its advance, in that the lesions developed on the mesocotyl were quite local, with definitely defined margins. (Cultures 43×49, 44×49.) Possibly these local lesions would have developed more extensively, but their appearance at the same stage of development on numerous plants of the same culture indicates that a definite resistance was being offered to the advance of the parasite.

There is no evidence in support of the statement of Dickson and Holbert (4) that susceptibility is dominant over resistance. Rather is it probable that the inheritance is quantitative, conditioned by multiple factors. The appearance of apparently resistant crosses in some few cases from parents of lower index indicates a possibility of obtaining resistant lines from susceptible strains.

Examination of F_3 lines from selected F_1 crosses: In order to gain a more extensive knowledge of the inheritance involved, certain crosses which exhibited marked differences between their respective parents were grown in the field in 1928, and the selfed progeny of each cross retained. Ears were selfed at random on F_2 plants grown in 1929. Thus, in 1929, a number of F_3 lines were available, 60 seeds from each of which were grown in the winter of 1929–30. The same procedure of planting in the greenhouse was adopted as for the parental lines and F_1 crosses and the same correction for germination made on the basis of the check plots. The crosses that were selected and the number of F_3 lines available from them in 1929 are as follows:

Cross	Supposed nature of cross	Number of lines available
43×46	R × S	60
43×47	R × S	90
49×50	S × I	45
58×60	S × S	70
64×66	I × S	31

Had the reaction of the F_1 cultures been known at the time of the selection of these crosses, others would doubtless have been added or substituted. For instance, 43×44 (R × S) was exceptionally resistant when tested in the F_1; 62×68 (S × S) also proved resistant in the F_1. This remains an avenue of investigation yet to be followed.

In order that a comparable basis for estimation of relative resistance of parental lines, F_1 crosses, and F_3 cultures might be available, the parental

lines and F_1 crosses that gave rise to the F_3 cultures were tested again at the same time. It has been stated already that this experimental series was carried out at a temperature of about 1° to 2° higher than that obtaining when the parents and F_1 crosses were first tested. Table 4 gives the results obtained, and by comparison with similar cultures of the parents and crosses in table 2 the influence of the difference in temperature is well evidenced. For convenience the results from the two trials are contrasted in table 3.

TABLE 3.—*Comparison between indices obtained from different cultures when grown at a temperature difference of 1–2° C. The first trial was at about an average temperature of 14° C., the second at about 15° C.*

Culture	Index obtained from 1st trial	Index obtained from 2nd trial
43	11	21
46	0	2
47	0	2
49	2	5
50	5	7
58	0	0
60	3	0
64	6	13
66	0	9
F_1 43×46	8	27
F_1 43×47	5	—[a]
F_1 49×50	12	29
F_1 58×60	2	8
F_1 64×66	9	27

[a] Seed not available.

It is obvious from the comparison that the indices of nearly all the cultures are increased as a direct effect of the rise in temperature. The increase is by no means proportionate, the crosses showing the greatest amount, while cultures 58 and 60 exhibit no increase whatever. It would seem that different cultures react differently to the same increase in temperature. Consequently, the variance between different cultures is increased at the higher temperature (15° C.) and this naturally is advantageous when the number of plants in each culture is restricted.

Table 5 is an analysis of the data presented in table 4. The F_3 lines are here arranged in 10 categories of descending degree of resistance as represented by the indices. In each category appears the number of F_3 lines of a similar index.

TABLE 4.—*Reaction of parents, F_1 crosses and F_3 lines originating from them, to Gibberella saubinetii. The reaction of each culture is represented by an index number. These numbers range from 0–30, and increase with the apparent resistance. Each index is given as the nearest whole number to that actually calculated*

Line	Index	Line	Index	Line	Index	Line	Index
43	21	40	19	33	29	88	8
46	2	41	14	34	23	89	9
47	2	42	4	35	23	90	5
49	5	43	23	36	26		
50	7	44	10	37	28	F_3 49 x 50	
58	0	45	7	38	19	1	11
60	0	46	12	39	30	2	8
64	13	47	18	40	16	3	10
66	9	48	27	41	21	4	11
F_1 43 x 46	27	49	23	42	25	5	10
F_1 43 x 47	.....[a]	50	15	43	29	6	16
F_1 49 x 50	29	51	12	44	18	7	5
F_1 58 x 60	8	52	15	45	20	8	16
F_1 64 x 66	27	53	13	46	19	9	2
		54	26	47	25	10	13
F_3 43 x 46		55	2	48	6	11	11
1	11	56	21	49	20	12	13
2	16	57	10	50	17	13	15
3	6	58	16	51	20	14	21
4	21	59	10	52	13	15	10
5	14	60	18	53	10	16	19
6	7			54	10	17	16
7	20	F_3 43 x 47		55	27	18	15
8	15	1	20	56	6	19	19
9	22	2	10	57	13	20	13
10	13	3	6	58	9	21	12
11	7	4	2	59	24	22	12
12	10	5	20	60	4	23	12
13	28	6	15	61	15	24	15
14	19	7	10	62	18	25	20
15	23	8	12	63	16	26	15
16	28	9	7	64	24	27	17
17	14	10	9	65	18	28	3
18	26	11	5	66	24	29	16
19	22	12	21	67	25	30	24
20	12	13	18	68	20	31	16
21	11	14	14	69	17	32	24
22	18	15	22	70	28	33	13
23	28	16	9	71	13	34	14
24	23	17	17	72	17	35	15
25	29	18	14	73	9	36	18
26	14	19	16	74	16	37	11
27	21	20	22	75	12	38	22
28	13	21	13	76	20	39	17
29	21	22	19	77	15	40	20
30	13	23	15	78	18	41	12
31	17	24	18	79	10	42	26
32	17	25	22	80	17	43	9
33	16	26	25	81	16	44	11
34	17	27	24	82	26	45	9
35	23	28	28	83	7		
36	10	29	18	84	22	F_3 58 x 60	
37	11	30	18	85	18	1	5
38	21	31	22	86	9	2	4
39	23	32	20	87	27	3	4

[a] Seed not available.

TABLE 4.—(*Continued*)

Line	Index	Line	Index	Line	Index	Line	Index
F_3 58 x 60		29	10	55	5	9	8
4	7	30	16	56	3	10	10
5	9	31	7	57	4	11	7
6	0	32	14	58	7	12	11
7	4	33	5	59	4	13	9
8	1	34	2	60	2	14	7
9	3	35	11	61	5	15	24
10	2	36	8	62	1	16	21
11	13	37	5	63	4	17	13
12	1	38	4	64	6	18	15
13	14	39	10	65	10	19	20
14	7	40	8	66	4	20	2
15	11	41	11	67	3	21	9
16	13	42	1	68	4	22	10
17	15	43	1	69	2	23	11
18	14	44	6	70	1	24	3
19	5	45	6			25	18
20	9	46	1	F_3 64 x 66		26	27
21	6	47	2	1	13	27	14
22	9	48	10	2	5	28	10
23	5	49	7	3	9	29	9
24	13	50	9	4	21	30	14
25	4	51	3	5	4	31	11
26	8	52	2	6	0		
27	4	53	1	7	8		
28	7	54	1	8	12		

Examination of this table will show that the distribution of the numbers in each class varies according to the type of original cross from which the F_3 lines originated and is in accordance with what might be expected from a Mendelian segregation of quantitative factors. Where a wide disparity exists between the degree of resistance of the original parents, as in 43×46 and 43×47, lines appear in the F_3 which also exhibit very considerable difference. Where the disparity between the parents is not so marked, but where they still possess an intermediate degree of resistance, as in 49×50 and 64×66, the F_3 lines also exhibit differences, but these are not quite so wide as in the two former cases. This may be partly, but not wholly, ascribed to the fewer individuals studied in the two latter examples. In the fifth cross, 58×60, where both parents are quite susceptible to the disease, no very resistant F_3 line appears, and indeed the modal class occurs at the lower end of the scale.

The distribution of these F_3 lines from each cross has been represented graphically in figures 2, 3, 4, 5 and 6. The solid line represents the curve obtained from the actual populations, while the dotted line represents the curve drawn when the populations are considered as 100 in each case. The position of the modal class will be seen to be nearer the lower end of the curve in all except one of the examples, viz, 43×47. The location of this

TABLE 5.—*Analysis of the reaction of parents, F_1 crosses, and F_3 lines, to Gibberella saubinetii. Indices are used as in tables 2 and 4. The F_3 lines are arranged in 10 categories of descending degree of resistance, i.e., in class frequencies*

Variety	Cross	Character of cross	Reaction of F_1	F_3 lines arranged in categories according to their reaction										Total number
				30–28	27–25	24–22	21–19	18–16	15–13	12–10	9–7	6–4	3–0	
Minn. No. 13	43 × 46	21 × 2	27	4	3	8	8	9	11	11	3	2	1	60
Minn. No. 13	43 × 47	21 × 2	....[a]	6	8	11	13	19	10	7	8	7	1	90
Minn. No. 13	49 × 50	5 × 7	29		1	3	5	8	10	12	3	1	2	45
Rustler	58 × 60	0 × 0	8					1	7	8	12	22	20	70
N. W. Dent	64 × 66	13 × 9	27		1	1	3	1	5	7	8	2	3	31

[a] Seed not available.

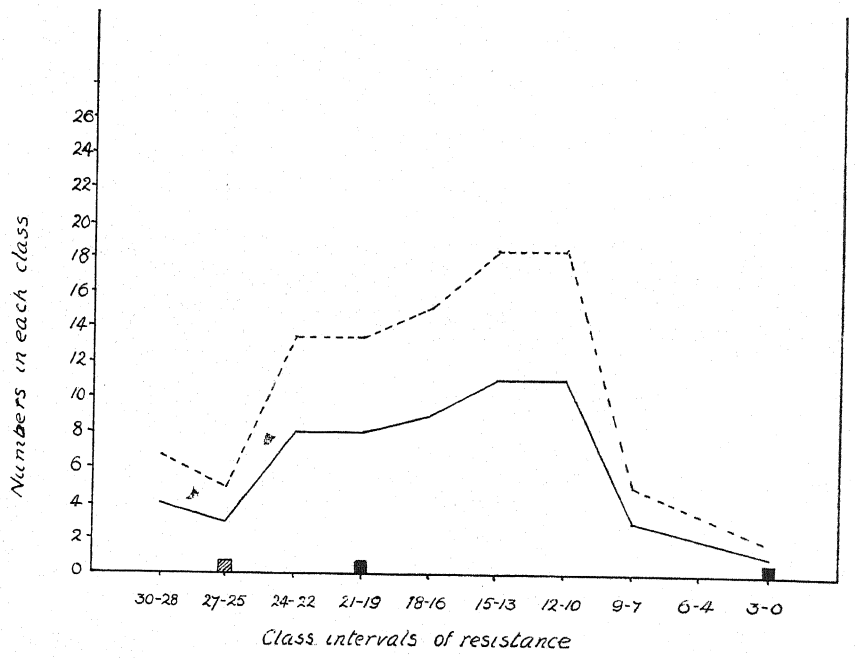


FIG. 2. Graphical representation of the relative distribution of resistance among the F_3 segregates from the original cross 43 × 46. The solid line represents the actual numbers (60), while the dotted line represents the population on a basis of 100. The two black squares indicate the classes into which the parents fall, and the shaded square the class into which the F_1 cross falls.

modal class should indicate which cross combines the greatest number of factors tending to increase resistance.

Where quantitative inheritance has been studied, it has been usual to find that parental types appear in later generations. In this study, it will be noticed that if the indices are interpreted literally, not only are the apparent parental types recovered, but segregates appear far in excess of the parents in resistance. The extent to which the parental lines would have varied had they been tested as extensively as the F_3 hybrids is unknown. Selfed lines do not exhibit the degree of resistance of the majority of crosses, and the indices of the selfed lines are to be regarded as merely indicating their *relative* resistance and are not to be compared directly with the indices of later hybrid generations. It would seem that resistance to *Gibberella saubinetii* is analogous to vigor in maize in that continued selfing isolates a range of segregates which do not approach the degree of resistance of the F_1.

In the first two crosses of Minnesota No. 13, viz, 43 × 46 and 43 ×47, a comparatively high proportion of resistant lines appears in the F_3 as well

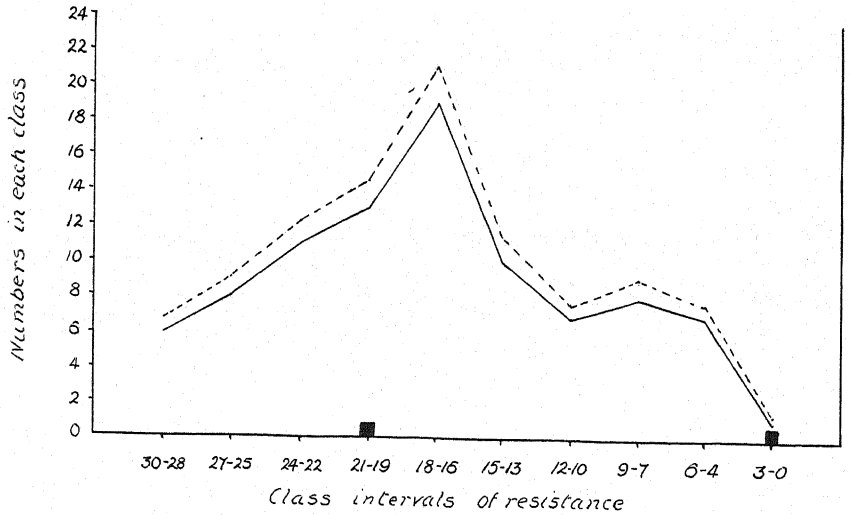


FIG. 3. Graphical representation of the relative distribution of resistance among the F_3 segregates from the original cross 43 × 47. The solid line represents the actual numbers (90), while the dotted line represents the population on a basis of 100. The two black squares indicate the classes into which the parents fall. In this case, seed of the F_1 was not available for testing.

as a number of quite susceptible lines. The uniformity of reaction of the plants composing each of these diverging lines points to their approach towards homozygosity in respect to disease resistance. Many of the lines of intermediate index also appeared uniform within themselves, but quite a few displayed marked differences in their individual constituent plants, from which it was inferred that segregation was in progress within the line. Such appearance of resistant and susceptible strains in a generation as early as F_3 might possibly be interpreted as indicative that the factors conditioning resistance are not numerous. But, recalling the easily variable nature of the reaction, such a postulate should be advanced with caution.

Observations on vigor and resistance: If resistance to root rot in the seedling stage is connected in some way with growth factors, then it might be supposed that vigor and resistance of seedlings would be correlated in some degree. Peterson (9) has denied any such relationship, and it was a matter of common observation during the present investigation to find, as did Peterson, strains that displayed much vigor but little resistance. Holbert *et al.* (7) have observed that seeds selected from diseased mother plants produce plants deficient in early vigor, even though infection of such seeds could not be detected by a germination test. There was opportunity to correlate mathematically the vigor of all the F_3 lines in the check bench with their respective indices of resistance in the inoculated material. The

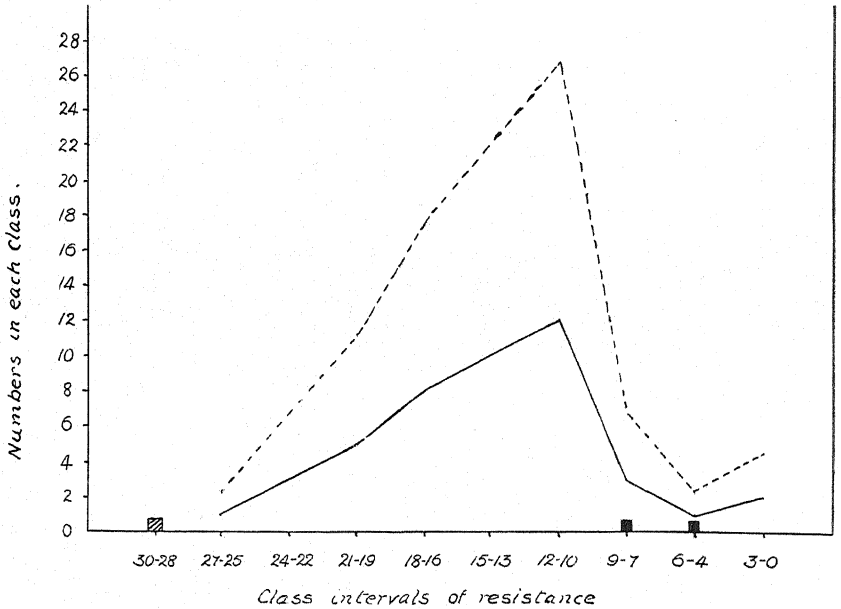


FIG. 4. Graphical representation of the relative distribution of resistance among the F_3 segregates from the original cross 49 × 50. The solid line represents the actual numbers (45), while the dotted line represents the population on a basis of 100. The black and shaded squares are used in the same way as in figure 2.

criterion of vigor was a scale of 1 to 5, in which the lower numbers represented low vigor, the high numbers greater vigor. Each plot in the check material was thus appraised, and, by adding together the two figures obtained from the corresponding plots, a range of values from about 3 to 10 was procured. These values were then correlated directly with the indices of reaction obtained from the inoculated cultures. Correlation coefficients were calculated for each of the five groups of F_3 lines, and these are presented below in table 6.

TABLE 6.—*Correlation between vigor of growth of seedlings in the check plots and reaction to Gibberella saubinetii in inoculated bench plots of F_3 cultures*

Variety and culture	Reaction of original parents	Average index of vigor	Correlation coefficients
Minn. 13 F_3 43 × 46	21 and 2	6.52	.199 ± .084
Minn. 13 F_3 43 × 47	21 " 2	6.27	.519 ± .052
Minn. 13 F_3 49 × 50	5 " 7	7.89	.155 ± .098
Rustler F_3 58 × 60	0 " 0	7.81	.107 ± .080
N.W. Dent F_3 64 × 66	13 " 9	7.45	.382 ± .104

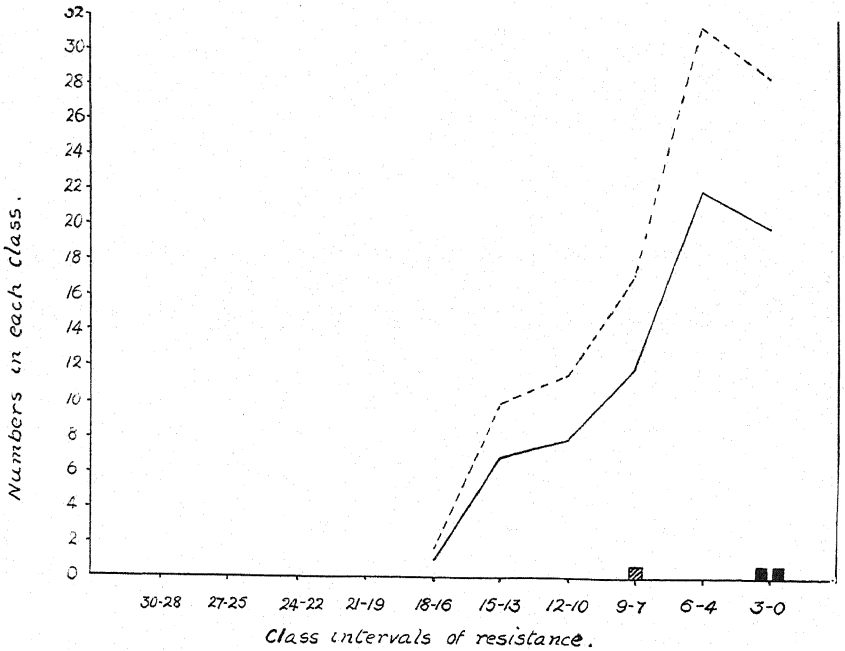


FIG. 5. Graphical representation of the relative distribution of resistance among the F_3 segregates from the original cross 58 × 60. The solid line represents the actual numbers (70), while the dotted line represents the population on a basis of 100. The black and shaded squares are used in the same way as in figure 2.

It is natural that in lines possessing little resistance correlation between vigor and resistance would be small. This is made apparent by the above table and exemplified by the two groups of F_3 lines from 49×50 and 58×60. On the other hand, these groups whose lines possess some degree of resistance tend to show significant correlations between vigor and resistance, *e.g.*, 43×46, 43×47, and 64×66. The groups are selected populations, and it is in accordance with expectation that such a result is found. Indeed, when all five groups were combined, the coefficient of correlation was found to be $-.002 \pm .039$, and, if the experiment were interpreted on this result, it would seem as if no correlation whatever existed. But to include such groups as 58×60 and 49×50 would not be logical, for factors for resistance are almost absent in them and no opportunity for variability exists.

While we are justified therefore in concluding that a definite correlation exists between vigor and resistance, the relation is by no means sufficiently constant to permit the practice of selection for resistance on the basis of seedling vigor.

The correlation between seedling reaction in the greenhouse to Gibberella saubinetii and yield in the field: If greenhouse testing of seedlings is to be

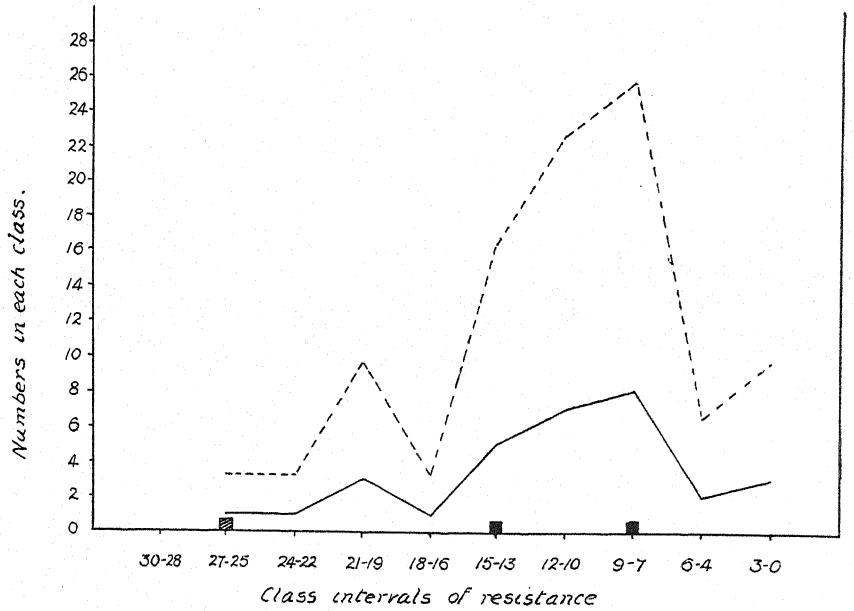


FIG. 6. Graphical representation of the relative distribution of resistance among the F_3 segregates from the original cross 64 × 66. The solid line represents the actual numbers (31), while the dotted line represents the population on a basis of 100. The black and shaded squares are used in the same way as in figure 2.

employed as a means of discriminating between resistant and susceptible lines, then it becomes of importance to know whether resistant lines thus delimited will show actual advantage over others when grown in the field. In short, we wish to know what advantage, if any, accrues to the breeding of corn which shows seedling resistance. Do resistant seedlings generally give higher yields, and do susceptible seedlings as a rule give lower yields? If this is the case, then we could expect to find a positive correlation between greenhouse resistance and yield in the field.

Holbert *et al.* (7) have found that "corn populations grown from seed . . . susceptible to the root and stalk rots produced a much lower yield of sound marketable corn . . . than corn grown from seed relatively . . . resistant to infection." On the other hand, Kiesselbach (8) has reported that "selection for freedom from root-rot disease by the germinator test does not increase grain production under the conditions of the experiment."

Field data upon yield taken in 1927 and 1928 were available from most of the crosses which had been tested for their reaction to disease in the greenhouse. These yields were computed from two systematically distributed replicates, each plot consisting of 12 hills, and bordered on both sides by the normal variety. Each hill was originally planted with 5 seeds,

and later thinned to 3. Only 2 and 3 plant hills were harvested, and these only when bordered on all 4 sides by 2 or 3 plant hills. Due allowance was made for the number of hills harvested, and the corrected yield expressed in percentage of the normal variety. These yields were computed primarily for the purpose of comparing the inherent yielding capacity of the F_1 crosses made at University Farm in connection with maize-breeding investigations.

It will be seen that with this method of computing yields, much of the influence of seedling injury upon the final yield is eliminated. For, in the first place, thinning to 3 seedlings is likely to cause retention of those seedlings which have escaped thorough infection, and in the second place, harvesting 2 and 3 plant hills would tend to eliminate the effect of those plants which died after thinning.

Consequently, by correlating directly the indices expressing resistance with the yields of the respective crosses, an opportunity to ascertain the effect of fungous invasion of the mature plants upon the yield of the crosses was afforded. In table 7 are given these correlations, with the yields of the separate varieties in 1927 and 1928 and also with the average yields of the same varieties for the 2 years. In addition, similar correlations are given when all 3 varieties are considered as one.

TABLE 7.—*Correlation coefficients between seedling reaction in the greenhouse and yield in the field*

Variety	1927	1928	Average of 1927 and 1928
Minn. No. 13	.317 ± .126	−.086 ± .107	.029 ± .141
Rustler	−.099 ± .138	.079 ± .116	.102 ± .139
N.W. Dent	.345 ± .127	.030 ± .147	.169 ± .155
Minn. No. 13, Rustler, N.W. Dent	.219 ± .077	−.024 ± .070	.103 ± .083

It will be seen from table 7 that the figures are contradictory. Only in 1927 do positive correlations of any magnitude appear, and, even in these, the probable error is so large (as in all cases) as to remove significance from the results. Even negative correlations appear in three cases. The only statement that can be made is that no correlation that is at all significant emerges from the data.

CONCLUSIONS AND SUMMARY

1. The investigation of the inheritance of maize to root-rotting organisms is fundamental to any rational method of producing resistant varieties. Owing to the nature of the disease, however, such investigation meets with experimental difficulties, and it was the purpose of this study not only to

attempt to elucidate the inheritance but to examine the methods employed and to appraise their dependability.

2. It is emphasized that uniformity of environmental influences, particularly temperature, is a desideratum if constancy of reaction to the disease is to be sought in any culture of maize. Not only should the temperature over the first 3 weeks of the experimental period be constant but equal distribution of temperature over the greenhouse should be assured. Especially is this latter condition to be fulfilled if dependable estimation of relative resistance is desired. The most suitable soil temperature at which to conduct the work was found to be about 15° C. When such a temperature was maintained, the correlation between replicates was sufficiently high (considering the small numbers used) to warrant drawing conclusions from the data.

3. Crosses of susceptible and resistant parents gave conflicting results in the F_1 generation. Sometimes a highly resistant hybrid resulted, and at other times the hybrid proved quite susceptible. Occasionally resistant hybrids appeared from parents possessing an intermediate degree of resistance, but in no case did very resistant progeny appear from parents of extreme susceptibility, although such crosses sometimes proved to be of intermediate resistance. It is impossible to state that resistance is either dominant or recessive to susceptibility. Rather is it more logical to assume that the inheritance is quantitative in nature and conditioned by multiple factors.

4. Examination of the F_3 progeny of 5 selected crosses gave further evidence of a quantitative inheritance. A definite segregation of F_3 lines occurred in conformity with a Mendelian explanation of such inheritance. Where the parental lines differed widely in reaction, highly resistant and susceptible lines appeared in the F_3, in sufficient proportion to suggest tentatively that perhaps relatively few factors are involved for intrinsic resistance.

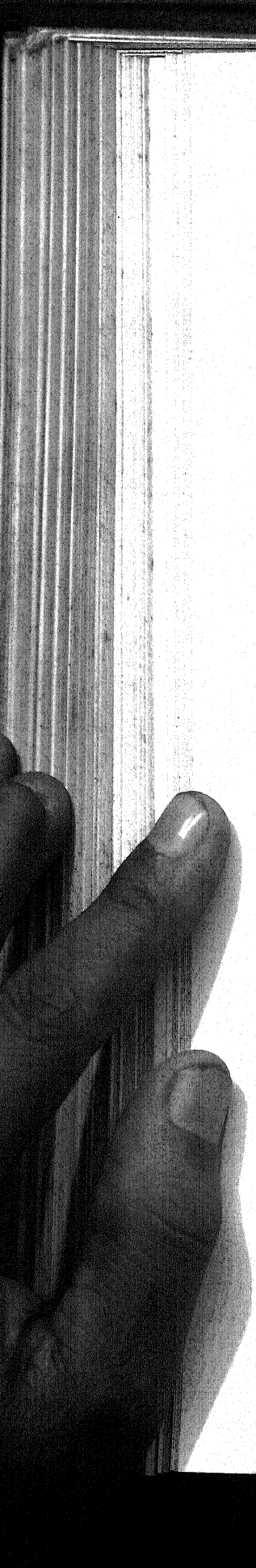
5. In F_3 lines obtained from parents differing widely in resistance, a definite and significant correlation was found to exist between seedling vigor and resistance, though the relation is not sufficiently constant to warrant selection for resistance on the basis of seedling vigor.

6. No significant correlation was found to exist between seedling reaction in the greenhouse and yield in the field as affected by fungous invasion of mature plants.

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BROWN SPOT OF TOBACCO CAUSED BY ALTERNARIA LONGIPES (E. & E.), N. COMB.

W. B. TISDALE AND R. F. WADKINS[1]

INTRODUCTION

The tobacco plant, whether grown for flue-cured, sun-filler, or shade-wrappers, is subject to leaf spotting caused by several different parasitic organisms throughout the Florida-Georgia tobacco district. Most of this leaf spotting, with the exception of wild fire and black fire, which are caused by bacterial organisms, may be attributed to different fungi. The most common fungi which may cause leaf spotting of tobacco of any consequence in Florida are *Phyllosticta nicotiana* E. & E., *Cercospora nicotiana* E. & E., and the species of Alternaria herein considered.

Because of the fact that leaves of bright tobacco are left on the stalks much longer than any other type of tobacco grown in this district before harvesting and therefore reach a more advanced stage of maturity, conditions for the development of brown leaf spot are more ideal. Hence, the brown leaf-spot disease is one that most commonly concerns the growers of bright tobacco. The information reported herein has been obtained from investigations of the disease on that crop, as the disease has been of little importance on cigar-wrapper tobacco.

THE DISEASE

In 1892 two species of Macrosporium were reported on tobacco from North Carolina (3). Since that year, various reports from Connecticut, Massachusetts, Missouri, North Carolina, Ohio, and Pennsylvania (1)[2,3,4] have been made, but in each case the disease and the organisms concerned seem to have been given very little attention. During the seasons of 1923 and 1924, Thomas reported[5] a species of Alternaria causing brown spots on tobacco leaves in Ohio. Isolations were made, but, as the disease was considered of minor importance, the cultures were discarded.[6]

[1] Formerly laboratory assistant, Tobacco Experiment Station, Quincy, Fla.

[2] Fant, G. W. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 45: 127. 1926. (Mimeographed.)

[3] Thomas, R. C. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 26: 143. 1923. (Mimeographed.)

[4] ———. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 34: 234. 1924. (Mimeographed.)

[5] *Op. cit.* (see footnote 4).

[6] Information contained in a letter from R. C. Thomas, dated February 5, 1929.

When the brown-spot disease first made its appearance in Florida is not known, but its occurrence did not become conspicuous until 1924, when flue-cured tobacco was introduced and grown on an extensive commercial scale (6). However, it is probable that the organism causing the disease occurred unobserved on cigar tobacco or on some other host plant prior to that time. Brown leaf spot usually makes its appearance each year during the latter half of July, occurring first on the sand leaves and spreading progressively upward to the top of the plant. The sand leaves are usually of poor quality and many growers leave them in the field, either on the stalk or on the ground. This practice may prove to be a source of later infection for the leaves higher up the stalk.

The first records of the disease in Florida were made by W. B. Tisdale, who observed it on flue-cured tobacco on a plantation in the southern part of Gadsden County, on July 24, 1925. Later, during the same growing season, the disease was observed in several widely separated areas. The disease now appears generally distributed over the entire Florida-Georgia district.

ECONOMIC IMPORTANCE

The total loss sustained each year by the growers of flue-cured tobacco as a result of brown spot depends largely upon the weather conditions and the cultural methods employed. Should premature ripening of the leaves occur as a result of insufficient moisture or from severe root-knot infection, the loss may be greater than under normal conditions. Also, improper cultural methods, such as late, deep plowing near the plants or delayed priming operations, may result in greater losses from the disease. However, the loss from brown spot may be considered somewhat minor as compared with the more important diseases of tobacco, such as wild fire, black fire, and black shank, when these occur. This is especially true under normal conditions when the leaves are harvested at the proper stage of ripeness. A crop of tobacco, severely spotted, will bring a much lower price than a crop that is comparatively free of spotting. The exact reduction in price per pound because of brown spot is not definitely known. If the infection is slight, the buyer often considers it an indication of ripe tobacco. However, it has been observed that a reduction in price was made on the Quincy market during the past 2 years for tobacco severely damaged from a leaf spotting caused by *Cercospora nicotiana* E. & E. and Alternaria. Therefore, it would be largely guesswork to attempt to give an estimate of the loss due to brown spot alone, since it develops under similar conditions and often simultaneously with the former organism. In 1925 Boyd[7]

[7] Boyd, O. C. U. S. Dept. Agr., Bur. Plant Indust., Plant Disease Rptr. Sup. 45: 127. 1926. (Mimeographed.)

reported a loss of 5 per cent of flue-cured tobacco in Georgia, which he attributed to a species of Macrosporium. In 1926, he again reported[8] the same disease in the South Georgia district. Boyd[9, 10] also has estimated the losses produced by *Alternaria* sp. for 1927 and 1928. Tisdale[11] reported the disease in Gadsden County, Florida, in 1927, but stated that buyers failed to make a reduction in price because of the disease except in cases of severe infection. It is difficult, therefore, to estimate the losses each year due to the Alternaria organism alone.

SYMPTOMS

The spots appear first on the lower leaves and enlarge rapidly. They often coalesce, thus rendering worthless the entire leaf area. As the leaves mature successively upward, spotting follows, and in severe cases, lesions appear on the stalks.

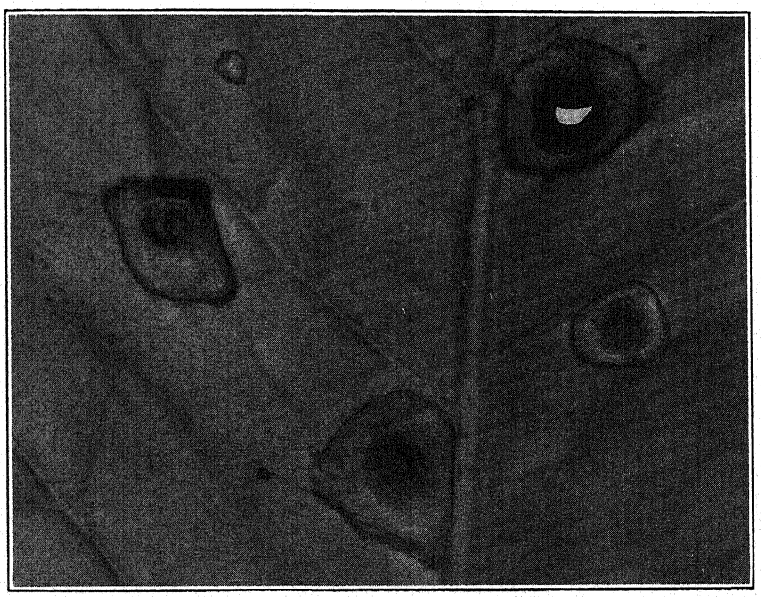


FIG. 1. Typical spots on White Stem Orinoco tobacco leaf produced by *Alternaria longipes* under field conditions. (Slightly enlarged.)

[8] ———. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 54: 316. 1926. (Mimeographed.)

[9] ———. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 61: 287. 1928. (Mimeographed.)

[10] ———. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. 12: 115. 1928. (Mimeographed.)

[11] Tisdale, W. B. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. 11: 138. 1927. (Mimeographed.)

The spots appear first as small, water-soaked areas which gradually enlarge, generally in a circular shape, unless checked on one side by the midrib or a prominent lateral vein. As the centers of the spots die and wither, they become sunken on both surfaces. The line of demarcation between the diseased and healthy tissues, under humid conditions, is not distinct, although, during dry periods, it becomes very pronounced. If damp weather should prevail over a prolonged period, there may be a halo margin or, in advance stages of the disease, varying blended shades from green to brown. The spots, under favorable conditions, may reach 1.5 to 2.5 cm. in diameter and may show a faint target-board effect or concentric rings (Fig. 1).

Lesions on the petiole are elongated and depressed and develop more slowly than those on the leaf blade. The lesions on the stalk may be numerous, somewhat sunken, and may finally girdle the plant. However, invasion never extends deeper than the cambium, and usually not that deep. Stem lesions usually do not produce conidiophores and conidia until the plant begins to decline.

Host Range

The information gained with reference to the host range of this particular fungus has been mostly from observations made in the fields at various times during the growing seasons of 1927 and 1928. Tobacco only has been observed to be affected with this organism. Several species of Alternaria have been isolated from various plants but, in each instance, the organism isolated proved nonpathogenic to tobacco.

Inoculation experiments under favorable temperature and humidity conditions with white potatoes, tomatoes, and peppers growing in pots and flats gave negative results.

Susceptibility of Different Varieties of Tobacco

In a test plot, where 24 varieties of bright tobacco were grown, progress of the disease was observed at intervals throughout the growing season. None of the leaves were harvested from the plot and the first leaves to become infected were left on the stalks. As the season progressed, infection developed higher up the stalks. It was observed that the sand leaves were the first to show the disease and by the time the seed pods were ready to be harvested nearly the entire leaf area of all varieties had become involved, with no indication of varietal resistance. The stalks also had numerous lesions throughout the entire length.

During the growing season of 1928, a small plot of ground near the laboratory was planted to the 10 following varieties of bright tobacco: Adcock, Big Stem Orinoco, Bonanza, Cash, Crutcher, Improved Warne, Improved

White Stem Orinoco, McAdoo, Narrow Leaf Orinoco, and Yellow Pryor. On July 9th a few of the lowest leaves of each variety were affected with brown spot, and by July 30th the entire leaf area had become involved and stem lesions were numerous. By the 20th of August most of the leaves had turned brown and had been shed. These observations indicate that none of the varieties of bright tobacco tested are resistant to the brown-spot disease under field conditions.

Inoculation Experiments

Numerous tobacco plants in all stages of development have been inoculated and kept under greenhouse conditions. Vigorous tobacco seedlings with 4 to 6 leaves, growing in flats and pots, have been atomized with water suspensions of spores on various occasions, but in every instance little or no infection developed. Because of this fact, it seems probable, therefore, that seedlings are not very susceptible to the disease, while in the plant beds, as long as normal rapid growth is maintained. However, if the plants are stunted, they become infected quite readily at any stage of growth. For nearly all inoculation work, plants from 12 to 20 inches high in flats or pots were used.

In all inoculation experiments, the plants were placed under moist chambers immediately after they were inoculated. The moist chambers were then covered with heavy wrapping paper or burlap bags to keep out the sunlight and to keep down the extremely high temperatures which develop under glass during the midday hours. Water was sprinkled on the plants through a nozzle from a water hose once every 24 hours for 3 or 4 days to maintain a high humidity, after which time the moist chambers were removed. The incubation period under these conditions varied from 5 to 8 days. If no infection was observed within this period, the plants were either discarded or no further observations were made.

Early in the spring of 1928 a series of inoculations was begun to determine whether there existed any degree of immunity among some of the varieties of bright tobacco grown in this district. Thirty plants of each of the following varieties were used for this inoculation work: Adcock, Big Stem Orinoco, Bonanza, Cash, Crutcher, Improved Warne, Improved White Stem Orinoco, McAdoo, Narrow Leaf Orinoco, White Stem Orinoco, and Yellow Pryor. When the plants reached 14 to 16 inches in height, 9 plants of each of the 3 groups of a variety were inoculated with a water suspension of spores from cultures 20 to 30 days old. One plant of each group was kept as a check by placing a sheet of paper between it and the 9 other plants that were inoculated. After 3 days, the moist chambers were removed and placed over 3 groups of plants of another variety that had just

been inoculated with a water suspension of spores. The inoculations of this series, therefore, followed each other at intervals of 3 days. The conditions were, however, essentially the same, except for the time element.

In summarizing the results obtained by inoculating the 11 varieties of tobacco, it was observed that there were some differences in the amount of infection produced. For the want of better terms, the following adjectives are used to describe the degree of infection: "severe," "medium," and "slight."

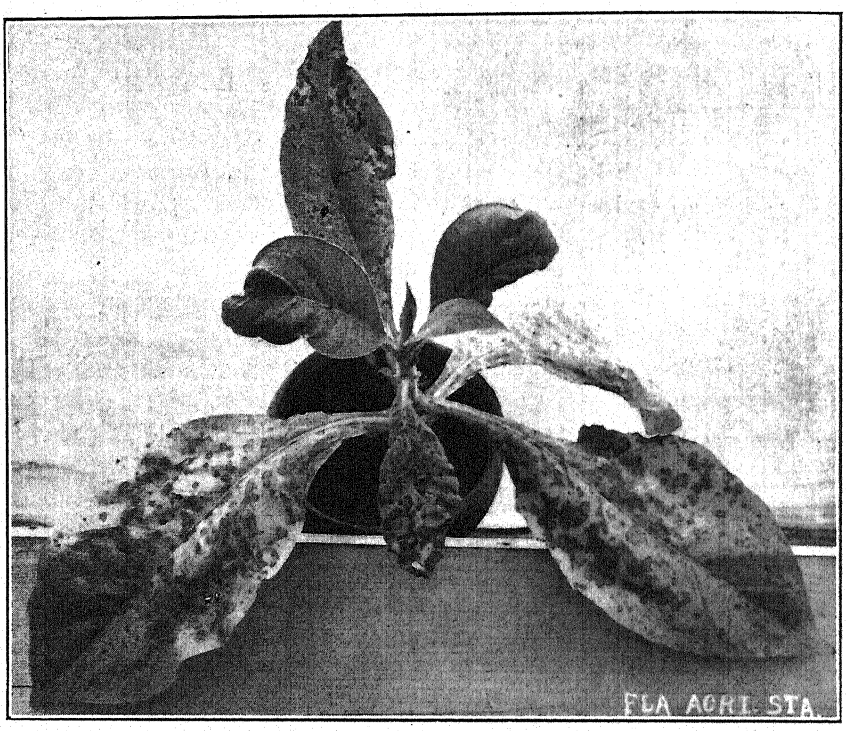


FIG. 2. Warne tobacco plant artificially inoculated with spore suspension of *Alternaria longipes*.

Infection was considered severe when all leaves except those near the bud were involved (Fig. 2); medium when the foliage halfway up the stalk was slightly spotted; and slight when only the 3 or 4 lower leaves were spotted.

Of a total of 27 Cash plants inoculated 23 developed severe infection. The other varieties which showed severe infection were Big Stem Orinoco, Improved Warne, Yellow Pryor, White Stem Orinoco, and Narrow Leaf Orinoco. The varieties which showed medium infection were Bonanza,

Improved White Stem Orinoco, Adcock, and McAdoo. Crutcher was the only variety on which slight infection was obtained. There were some individual differences as between groups of plants of each variety, but the results shown represent the average for the variety. To judge from the results of this series of inoculations, there does not appear to be enough resistance in any one variety tested to enable a grower to escape all losses from brown spot. Figure 3 well represents the effect of Alternaria on the entire collection of plants inoculated.

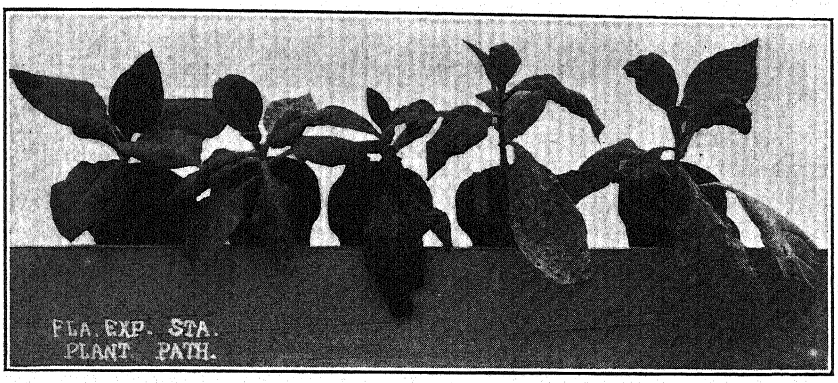


FIG. 3. Tobacco plants inoculated with *Alternaria longipes*. From left to right: Check plant (Connecticut Round Tip), Big Cuba, Big Stem Orinoco, Yellow Pryor, and Warne.

THE FUNGUS

Overwintering

One of the chief ways that this species of Alternaria appears to overwinter is on the old stalks left standing in the fields. Early in the spring of 1928 isolations were made from portions of old stalks known to have been heavily infected the previous growing season. Three different species of Alternaria were obtained from this material but only one of them proved pathogenic to tobacco. In August, 1928, 24 stalks bearing brown-spot lesions were brought from the field and stood upright in a trench near the laboratory building. On the same date several other infected stalks from the same source were cut into sections 10 to 12 inches long, wrapped in thick paper, and carried through the winter under the following conditions:

Lot No. 1 buried 6 inches deep in the soil.
" " 2 " 4 " " " " "
" " 3 " 2 " " " " "
" " 4 placed on the surface of the ground.
" " 5 suspended 2 feet in the air from a wire fence.

Isolations were attempted from time to time during the winter of 1928–29 from the stalks on the surface of the ground, the ones standing upright and the ones suspended aboveground. In almost every attempt, a species of Alternaria was obtained that was very similar in growth characters to the one found during the growing season.

The stalk material buried underground gave negative results when isolations were attempted. All of this material was in a partial state of decay when first examined a month after being placed in the soil. By the 11th of February, 1929, all the stalks were in an advanced state of decay and were discarded.

Spores could be seen on the lesions throughout the winter on the old stems carried over the winter in the open. This was especially true during warm, cloudy periods accompanied by rain. The temperature at such times was high enough to cause the old mycelium in the tissues of the old stalks to send out new conidiophores and produce conidia. Some of the spores produced under these conditions were irregular in size and shape. The younger spores were of the type found on the leaf lesions during the summer months, while the older ones were irregular in shape and deeply constricted at the septa. This was also observed by Elliott (2) in his studies on the genus Alternaria. These longevity studies show that sufficient viable spores are present at planting time to produce primary infection. Temperature studies of the organism in culture, as given elsewhere, show that vegetative growth and spore production occur at low temperatures.

Proof of Pathogenicity

Isolations were made from spotted leaves by W. B. Tisdale in the summer of 1925. During February and March of 1926, artificial inoculations with water suspensions of spores were made on plants in the greenhouse under moist chambers. In one flat, the lower leaves had been stunted and were somewhat yellow at the time of inoculation. After 5 to 7 days, numerous small brown spots were evident and the fungus was reisolated from the diseased areas. No further inoculations were made until December, 1926. During that month 3 Connecticut Round Tip tobacco plants, 2 feet high, were atomized with a water suspension of spores and placed under a moist chamber for 5 days. Although several leaves were punctured in one or more places with a sterile needle before they were atomized, no infection developed.

In February, 2 flats containing Connecticut Round Tip plants were inoculated with a water suspension of spores and placed under moist chambers for 3 days. At this time a number of small, incipient, water-soaked areas were observed. After 8 days numerous brown lesions had developed

on the leaves, some of which had coalesced. An Alternaria was isolated from these lesions, similar in every respect to the original organism. During February, 1927, the temperature was somewhat higher than in December, 1926, when the first inoculation was made. This may account for the negative results of the previous inoculation. Numerous other inoculations were made during the summer months and infection always developed when the plants were in a humid atmosphere. Rands (5) reported that good infection of early blight of potatoes occurred during days of high humidity and high temperature.

Effect of Temperature on the Degree of Infection

In order to determine more accurately the effect of temperature on infection, tobacco plants were inoculated at different seasons of the year and records of the temperature and humidity were kept during the incubation period. In the winter of 1927–28, a series of inoculations was begun and continued until midsummer of 1928. For this work only plants of the Cash variety were used. The relative humidity under the moist chambers in these experiments was approximately 90 per cent during the 3 or 4 days following each inoculation. This humidity was high enough to keep the foliage and glass of the moist chambers moist. The inoculations made in midwinter when the temperature was relatively low gave almost negative results, while the ones made later, when night temperatures were higher, resulted in more severe infection. The degree of infection apparently depended on the temperature for the 3- to 5-day period following the time of inoculation, thus indicating that temperature is an important factor in infection. When the average temperature was 19° C. and below, no infection was produced. As the average temperature gradually climbed to about 20.5° C., slight infection was produced on the older leaves. Medium infection was secured with mean temperatures of 23 to 25° C. and severe infection was obtained when the mean temperature was from 26.5 to 31.0° C.

There is a close relation, it appears, between the temperature at which infection developed and the mean temperatures for the months of January to August, 1928, inclusive. The mean temperature for the months of the first half of the year are as follows: January, 10° C.; February, 14° C.; March, 17° C.; April, 17° C.; May, 22° C.; June, 26° C.; July, 27° C., August, 27° C. By comparing the temperatures at which medium or severe infection was produced experimentally, with the mean temperatures for June, July, and August, one would expect abundant field infection during these months. Observations extending over a period of several years have shown that these are the months during which the disease occurs and develops in the fields. Therefore, the severity of the disease appears to be inti-

mately associated with the degree of maturity of the plant and relatively high humidity and temperature.

Method of Infection

A water suspension of spores was applied to marked portions of leaves with a loop needle and these inoculated areas were killed, clarified, and examined to determine the relation of parasite to host. After 12 to 18 hours' incubation at room temperature, these portions of the leaves were cut out and placed in Gilson's solution for 18 to 20 hours, then washed thoroughly in running water and placed in a weak solution of alcohol. This material was run through the alcohols and xylol and, after remaining in pure xylol for 3 to 4 days, the leaf tissues became transparent enough to permit light staining with eosine-lactophenol. This process permitted a view of the germinating spores and the germ tubes penetrating the epidermal cells. It was found that 1 to 4 germ tubes had been produced and these were entering the leaf through the stomata. A few germ tubes were observed entering the leaf through the basal cells of epidermal hairs and directly through the epidermis.

Morphology

Mycelium. The mycelium permeates the leaf tissue in all directions from the point of infection, passing between and through the cells. The hyphae vary in diameter on different substrata, ranging from 1 μ in the plant tissue to 4.5 μ in culture media. The older hyphae are much larger in diameter than the younger ones and have more septations. The cell walls are thicker in the older growth. In the leaf tissue the hyphae soon come near the surface and either break through the epidermis or emerge through the stomata. At this stage, the cells of the host tissue collapse, resulting in sunken brown lesions. In this condition when accompanied by high humidity, numerous conidiophores, 3 to 10, develop at one point, and produce conidia. In cultures 10 to 15 days old the entire growth becomes dark brown or olivaceous, producing an abundance of conidia.

Conidia. The young conidia are of the same color as that of the imbedded hyphae. They are ovate and slightly constricted at the septa. The cell walls become thicker and constrictions at the septa become more pronounced as the spores grow older. Conidia on the host may be much larger than those grown on culture media. From leaf spots they measure 11–13 × 30–86 μ, averaging 12 × 50 μ. The number of cross septations varies from 3 to 5, with a good percentage of spores having 1 to 2 longitudinal septations (Fig. 4, A). Spores from 20-day-old cultures, on potato-dextrose agar, measured from 6.5–13 × 25–52.6 μ, the average being about 9.6 ×

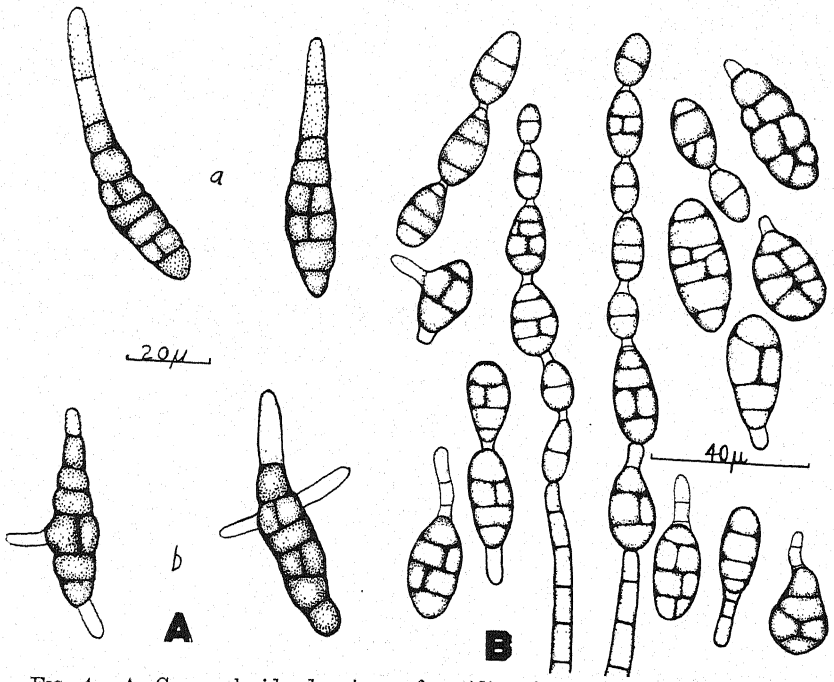


FIG. 4. A. Camera-lucida drawings of conidia of *Alternaria longipes* from host plant: a. As they appear under field conditions; b. Germinating. B. Camera-lucida drawings of conidia of *A. longipes* produced on potato-dextrose agar, showing different types and chains.

29.3 μ. The number of septations in most spores from culture media is about the same as that found on the host plant, although a few spores have only 2 or 3 cells.

Taxonomy

Ellis and Everhart (3) reported a "white speck" of tobacco from North Carolina in 1892 caused by *Macrosporium tabacinum,* n. sp. The description of this disease and causal organism as given by them is as follows:

Spots amphigenous, numerous, thin, white (rusty red or brown at first), suborbicular or irregular, 2–3 mm. in diameter, definitely limited, with a narrow darker border. Fertile hyphae effused, 35–45 × 3–4 μ, septate and torulose above. Conidia obovate, 15–25 × 10–12 μ sessile, or longer (35–45 μ) narrowed below into a distinct stipe, 8–12 μ long. The shorter conidia are mostly 3-septate and the longer ones about 5-septate, one or two of the cells with a longitudinal septum. This is the "white speck" of the North Carolina planters.

In this same reference, Ellis and Everhart described *Macrosporium longipes,* n. sp. from North Carolina as causing "brown spot" of tobacco. The description of this species is as follows:

Spots amphigenous, orbicular, rusty brown, 3–5 mm. in diameter; orbicular, zonate. The entire leaf becomes brown and then the spots are a shade lighter than the surrounding parts. Fertile hyphae effused on the spots, amphigenous, but more abundant above, slender (40–70 × 3–4 μ), septate and often constricted at the septa; erect, more or less torulose above. Conidia clavate, 40–50 × 15–20 μ, 3–7, mostly 5–6 septate, with two or more of the cells divided by a longitudinal septum, attenuated below into a distinct stipe 35–50 μ long, and often septate and torulose. This differs from *M. commune* Rabh., in its effused hyphae and smooth conidia, and from *M. tabacinum* Ell. and Ev., in its brown, concentrically zoned spots and larger stipitate conidia. Known among the planters as "brown spot."

No mention is made in literature of any inoculation experiments with either of these organisms and, as a result, their pathogenicity has been questioned (4). These two reports appeared many years ago and no recent reports of work with Alternaria or Macrosporium on tobacco have been found.

The description of the species of Alternaria found on tobacco in the Florida district is as follows:

Spots amphigenous, orbicular or slightly irregular, older ones 1.5 to 2.5 cm. in diameter, dark brown center, bordered by a band of lighter color. A narrow dark brown band or ring separates this lighter colored band from the green healthy tissue. Center of spots never turns white. Faint concentric rings are apparent in some spots (Fig. 1). Many spots are never larger than 3 to 10 mm. in diameter. When spots are numerous, coalescence takes place and a large portion of the leaf becomes brown. Fertile hyphae effused on the spots, somewhat erect, more abundant above, 35–74 × 3–4 μ. Conidia clavate to ovate, often with short beaks with 1–3 septations, 3–7 septate, one or two cells longitudinally septate, slightly constricted at the septa, 30–50 × 10–13 μ (Fig. 4, A).

In culture on potato dextrose agar the margin of growth is regular or slightly dentate: young mycelium white, turning olive green to black with age; aerial hyphae abundant, 5–7 mm. high, fluffy or tufted, bearing numerous conidiophores with catenulated spores (Fig. 4, B). Spores 3–5 septate, a few 1–2 longitudinally septate, 24–25 × 7–13 μ, slightly constricted at the septa, becoming more pronounced with age. In 30-day-old cultures, the color is dark olivaceous or almost black.

There is no exact way of determining at present the exact relation that may exist between the two species of Macrosporium reported by Ellis and Everhart (3) and the species of Alternaria found on tobacco in Florida. Some of the species of Macrosporium have been shown to produce catenulated spores, which, therefore, necessitated placing them in the Alternaria group. The only method of comparing either of Ellis and Everhart's organisms with the one considered in this paper is by considering spore measurements and the characteristics of the disease produced by each. In

comparing the spore measurements, one is likely to arrive at the wrong conclusion, since it has been shown by Elliott (2) that the dimensions and other morphological characters of spores of Alternaria in *exsiccati* may vary considerably. Then, again, the measurements of spores of the tobacco Alternaria found in Florida produced some smaller spores on culture media (potato-dextrose agar) than those found on the host plant. In considering the characters of the disease, it appears still more confusing, for some of the characters produced by each organism very closely resemble each other, while, on the other hand, there is one outstanding difference. The white spots produced by *Macrosporium tabacinum* E. & E. are not found with either *M. longipes* E. & E. or *Alternaria* sp. on Florida-grown tobacco.

In table 1, the spore and spot characters are summarized. It will be observed that spore measurements of the Alternaria herein described more closely resemble those of *Macrosporium longipes* E. & E. According to Ellis and Everhart (3), the principal differences between *M. longipes* and *M. tabacinum* are in the size of the spores and the color of the spots.

It appears from table 1 that the differences between the organism under consideration and *Macrosporium longipes* E. & E. are not sufficiently great to warrant a new species. However, because the spores are produced in chains, the new combination **Alternaria longipes** (Ell. & Ev.) is suggested.

TABLE 1.—*Summary of the primary characteristics of spores of and the diseases caused by Macrosporium longipes, M. tabacinum, and Alternaria longipes*

Organism	Spore measurements	Spore characters			Spot characters
		Cross Sept.	Longi. Sept.	Spore shape	
M. tabacinum	15–25 × 10–12 μ	3–5	1–2	Obovate stipitate	Amphigenous, red or brown later turning white.
M. longipes	40–50 × 15–20 μ	3–7	1–2	Clavate stipitate	Amphigenous, brown, zonate.
Alternaria longipes	30–50 × 10–13 μ	3–7	1–2	Clavate to ovate	Amphigenous, brown, zonate.

Physiology:

Cultural Characteristics

During the progress of this work *Alternaria longipes* has been grown on several kinds of agar culture media and on cooked stems of tobacco, sweet clover, potato, tomato, and bean pods for comparison and to determine whether a perfect stage would develop. In the course of study the following observations were made:

Potato-dextrose agar. Single germinating spores when transferred to Petri plates of this medium at room temperature produced a light olivaceous growth, measuring on an average 23 mm. in diameter by the third day. After 10 days, the increase in diameter averaged 6.25 mm. per day. Nearly all single-spore cultures in Petri dishes show a distinct zonation or concentric bands of light and dark color. After a few days dark sectors develop (Fig. 5) and, later, the entire colony becomes dark olivaceous. Aerial hyphae are abundant, somewhat in tufts or mats upon which spores are produced in abundance in cultures 5 to 7 days old.



FIG. 5. Growth of *Alternaria longipes* on potato-dextrose agar at 22° after 8 days.

Oat agar. Growth on oat agar produced scant aerial hyphae, margins regular, thin and light colored, and spores relatively abundant at the end of 5 days. Single-spore plate cultures increased in diameter at the rate of 9.5 mm. per day.

Plain potato agar. The rate of growth was about the same as that on potato-dextrose agar but a much longer time was required for sporulation.

Tobacco-dextrose agar. This medium was prepared as follows: 200 gm. of tender tobacco stems were added to 500 cc. of distilled water and cooked in a double boiler for 45 minutes. To this decoction 17 gm. of bacto-agar and 20 gm. of bacto-dextrose were added and the entire volume was brought up to 1 liter, cooked, and autoclaved. The color of aerial hyphae produced on this medium was almost black. Daily increase in diameter of growth was 4 mm. Spores were very dark in color and abundant at the end of 7 days.

Sweet-clover agar. This medium was made up by using 200 gm. of sweet clover stems, 17 gm. of bacto-agar, and 20 gm. of bacto-dextrose to 1 liter of distilled water. The rate of growth and other characters produced on this medium were about the same as those observed on potato agar, except for lighter colored aerial hyphae.

Sterilized sweet-clover stems. (In Roux tubes with water and absorbent cotton on the bottom.) Growth was rapid, covering the stems in 5 to 7 days, with light olive-green growth, later turning much darker. Spores were relatively abundant at the end of 10 days. The fungus had grown to the bottom of tubes after 90 days and was producing spores on the cotton.

Sterilized bean pods. (Prepared in Roux tubes.) The growth of mycelium was vigorous, almost white at first, later turning olivaceous, and producing abundant spores at the end of 10 days.

Sterilized potato stems. (Prepared in Roux tubes.) Growth was somewhat slow, whitish at first, but, later, turning to an olive green. Spores were produced in abundance in 10 to 12 days.

Throughout all the cultural work conducted in the laboratory no evidences of a perfect stage were observed on any culture media used. The same type of spore was produced on all culture media, except that those produced on oat agar were slightly smaller, averaging $10.8 \times 25.2\ \mu$.

Effect of Temperature on the Development of the Fungus in Culture

In preliminary tests it was found that single-spore cultures made the greatest growth in diameter at pH 5.0 to 6.5, at a temperature of 24 to 27° C. Using this as a basis, all cultures were adjusted to pH 6.5 by the addition of N/1 hydrochloric acid. Only potato-dextrose agar was used for this particular work. The spores were germinated in dilution plates of potato agar kept at 23 to 24° C. and those which showed as near the same stage of germination as could be obtained were used to inoculate sterile Petri plates. All cultures for temperature studies were run for 8 consecutive days. The cultures from 25 to 35° C. were kept in a Freas oven with the temperature varying not over 1 or 2°. Cultures kept at 29–31, 32–33, and 34–35° C. were placed in a moist chamber to prevent rapid desiccation of the culture media. Cultures exposed at from 10 to 25° C. were kept in various places in a refrigerator. Under this condition, fluctuations in temperature were greater than with cultures placed in the Freas oven at higher temperatures.

Table 2 shows that the fungus grows in culture media over a fairly wide range of temperature. The optimum temperature for growth, based upon rate of increase in diameter of colony, lies between 25 and 29° C. The decrease in rate of growth is very rapid from 29 to 35° C. where no growth occurred. After germinated spores had been kept at 35° C. for 24 hours

they failed to make any growth in 3 days when transferred to another incubator kept at 25° C. Mature spores in dilution plates containing potato-dextrose agar germinated within 5 hours at 35° C., but when the germ tubes attained a length about equal to the diameter of the spore no additional growth occurred. From the optimal temperature down to 10 degrees, the decline in growth was more gradual. Because of the lack of facilities, the minimal temperature could not be determined but, judging from the growth made at 9–11°, it is apparently not much below 9° C. Spores of the usual size and shape were produced in abundance near the optimal temperature. Sporulation also occurred in cultures kept at higher temperatures, but the spores were smaller and more irregular in shape. Spore formation was retarded toward the lower end of the temperature range.

TABLE 2.—*Diameter of single spore cultures of Alternaria longipes on potato-dextrose agar exposed for 8 days at temperatures indicated*

Temperature °C.	No. plates	Average diameter of growth in mm.
9–11	20	8.3
11–13	22	13.5
12–14	24	18.7
14–16	24	25.3
17–19	25	33.6
18–21	20	41.2
21–23	24	49.2
25–26	35	53.2
26–28	30	58.0
29–31	35	25.0
32–33	45	9.8
34–35	45	0.0

The range of temperature for spore germination is almost identical with that for vegetative growth and sporulation. About 53.2 per cent of the spores germinated at the end of 36 hours at 9–11° C. Only 1 or 2 germ tubes developed at this temperature and 24 to 28 hours were required for them to attain a length equal to the diameter of the spore. At 11–14° C., the time for germination was 20–22 hours with 59.6 per cent germination. From 16–20° C., the time for germination was reduced to 14 to 16 hours

with 73.4 per cent germination. At temperatures ranging from 21–25° C., 78.3 per cent germination was secured within 10 hours. The most favorable temperature for germination was between 26–29° C., where 93.4 per cent of the spores germinated within 8 hours. From 30–35° C., the time for germination was reduced to 5 hours with practically all the cells of the spores sending out germ tubes. The percentage of germination at these higher temperatures was not appreciably different from the figures obtained at 26–29° C.

Effect of Hydrogen-ion Concentration of Culture Media upon the Rate of Growth of Alternaria longipes

Potato-dextrose agar was used in the experiments to determine the effects of hydrogen-ion concentration upon the rate of growth of *Alternaria*

TABLE 3.—*Growth of single-spore cultures of Alternaria longipes in 8 days at 26° C. on potato-dextrose agar of pH concentration indicated*

pH value of culture media	Growth in diameter—mm.	Growth characteristics
1.50	No growth	
2.00	Very little growth	White, warty growth. No spores produced.
2.50	3.2	White, warty growth. No aerial mycelium produced. Becomes sooty white with age. No spores.
3.00	7.5	Sooty white, very little aerial mycelium. Spores few and irregular in shape.
3.50	13.6	Growth of grayish color, aerial mycelium abundant. Spores more abundant than at pH. 3.00.
4.00	20.1	Growth almost black in color, aerial mycelium abundant. Spores abundant. Margins regular.
4.50	45.0	do
5.00	50.4	do
5.50	58.5	do
6.00	55.2	do
6.50	43.7	do
7.00	48.5	Color typically olivaceous. Margins more or less irregular. Aerial hyphae relatively abundant.
7.50	54.0	do
8.00	45.8	Growth much lighter in color. Margins almost regular. Spores abundant, regular in shape and color.
8.50	42.3	do
9.00	31.6	do

longipes. The cultures were run in quadruplicate at each pH reaction and the experiment was repeated. The measurements shown in table 3 are the average for the two series. Two liters of potato-dextrose agar containing 2.2 per cent bacto-agar were prepared for each series. The medium was divided into the several lots to be adjusted to various pH values, ranging from pH 1.5 to pH 9.0 by the addition of N/1 hydrochloric acid and N/1 sodium hydroxide. Determinations were made by the use of a quinhydrone potentiometer. Petri plates were inoculated in the same manner as that described in temperature studies on culture media. The plates were then exposed to a constant temperature of 26° C. for 8 days. At the end of this period, measurements and observations were made and are summarized in table 3.

From these results it will be observed that, so far as diameter of growth is concerned, the most favorable reactions were obtained with pH values between 4.50 and 8.00.

CONTROL MEASURES

In discussing control measures for brown spot of tobacco, it will be well to keep in mind that work done towards prevention is far more important than trying to check the disease in a field after the plants once show signs of infection. Since spraying or dusting the plants would add considerable expense in both labor and materials to a crop that is more or less uncertain in its returns and since spray or dust materials are objectionable on the cured leaf, it seems more advisable to resort to sanitary measures and improvement of cultural methods. When harvesting is complete, the stalks should be cut with a stalk cutter and turned under to bring about complete decay as quickly as possible. If these operations are inconvenient just after harvesting the top leaves, the stalks may be cut and turned under later in the season.

Crop rotation might be used advantageously in combating the disease, because it will not only reduce to a minimum the chances for primary infection of brown spot but will also reduce the chances for infection by other parasites. Since brown spot has been observed to be serious on plants affected with root knot, it is advisable to adopt a rotation system which will reduce root-knot infection to a minimum. Cultural methods should be so regulated as to keep the plants in a vigorous state of growth until they are mature; then, the leaves should be primed as they become ripe or mature. Most of the natural infection has been observed to occur on overripe leaves or on leaves that have been prematurely ripened because of root knot or insufficient water supply.

SUMMARY

1. A leaf-spot disease of bright or flue-cured tobacco, commonly known as brown spot, has been prevalent for several years in the Florida-Georgia tobacco district.

2. A similar, if not the same, disease was reported by Ellis and Everhart in 1892 from North Carolina.

3. In comparing reports of losses caused by various tobacco diseases in this district, it will be noted that, while the brown-spot disease is not of major importance, yet, during certain seasons when ideal conditions prevail for the activity of the fungus, considerable reduction in both price and yield is sustained by the growers.

4. The disease appears first on the lower leaves as small spots which rapidly enlarge and become brown. If the spots are numerous, coalescence takes place and the entire leaf may turn brown. Under very favorable conditions the disease spreads rapidly to the upper leaves and causes a complete loss of the crop.

5. So far as has been observed in fields and also from inoculation experiments, tobacco is the only plant susceptible to infection.

6. There appears to be very little difference in the susceptibility of the different varieties of bright tobacco tested.

7. The organism hibernates on the stalks left standing in the fields or lying on the surface of the ground.

8. Proof of the pathogenicity of the organism has been fully established by numerous inoculation experiments.

9. The mode of infection is by direct penetration of the epidermal cells and through the stomata, wounding being unnecessary for infection.

10. The organism herein reported produces catenulate spores, comparable in size to those of *Macrosporium longipes* E. & E., and the character of the spots is almost identical. Because of catenulate spore formation, however, the name **Alternaria longipes** (E. & E.), new combination, is proposed.

11. The minimal temperature for infection is about 20 to 22° C. Medium infection was produced when the average temperature was from 23 to 25° C. Severe infection was produced when the average temperature was 26.5° C. and above. The maximal temperature for infection was not determined.

12. The optimal temperature for the development of the fungus on culture media was between 25 and 28° C. The maximal temperature is near 34–35° C. The minimal temperature for the development of the fungus in culture was not determined.

13. The optimal temperatures for most severe infection and for growth of the fungus in culture are approximately the same.

14. Maximal amount of growth on potato-dextrose agar was secured at pH 5.5. There was a slight decrease in growth with pH values 6.0 and 6.5 but an increase again with pH 7.0 and 7.5.

15. The stalks should be cut and turned under immediately after the crop is harvested to minimize the source of inoculum the following year. Good fertilization, together with crop rotation and frequent cultivation, is advisable. The leaves should be primed when they reach the proper stage of maturity.

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FURTHER STUDIES ON REACTION OF CORN TO SMUT AND EFFECT OF SMUT ON YIELD[1]

F. R. Immer and J. J. Christensen

INTRODUCTION

Breeding for disease resistance seems to be the only promising method for controlling corn smut, *Ustilago zeae* (Beckm.) Ung. The chlamydospores are produced in enormous numbers and can live in the soil for several years; therefore, inoculum will always be present in abundance in the corn-growing area. Part of the life cycle of the organism can be spent as a saprophyte in the soil and part as a parasite on corn plants. Adequate control by cultural practices is, therefore, practically impossible.

It has been shown that inbred lines of corn, quite resistant to smut, can be produced rather easily. The rapidity with which these selfed lines become homozygous for reaction to smut suggests that a relatively small number of genetic factors are involved in determining smut reaction. Breeding for resistance to smut by selection in self-fertilized lines seems to be the most promising method for controlling the disease.

REVIEW OF LITERATURE

Extensive tests have demonstrated that inbred lines of corn which differ markedly in resistance to smut (3, 5, 7, 8, 9) can be produced. The reaction of these inbred lines to smut infection has been found to be quite constant from year to year. Inbred lines can be produced with every degree of resistance or susceptibility, when grown in an epiphytotic of the disease. Kyle (13) has shown that a tight husk covering favors resistance to ear infection.

Several controlled experiments have been conducted to determine the damage caused by smut galls on parts of corn plants other than the ears. Analyses were made on the basis of barrenness induced by the fungus and reduction in yield of shelled corn caused by smut galls on the plants. Garber and Hoover (4) found that smut infections increased the percentage of barren plants, but there was no significant decrease in yield due to smut infection apart from the greater percentage of barren stalks. Immer and Christensen (10) and Jorgenson (12), working in Minnesota and Ohio, respectively, used essentially the same methods and found that smut galls on the plants of inbred lines reduced yields very materially. Jorgenson found essentially the same condition in F_1 crosses.

[1] Contribution from the Division of Agronomy and Plant Genetics and the Division of Plant Pathology and Botany, University of Minnesota, St. Paul, Minnesota.

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It has been demonstrated in a number of instances that smut collections from different localities differed in their ability to cause infections when the plants were inoculated with a hypodermic syringe or when inoculum was dropped in the apical heads of young corn plants (1, 2, 6, 17). Smut collections made in different States or in different parts of the same State or locality have sometimes been found more virulent than local smut collections under these conditions. No extensive tests have been reported previously giving the reaction of corn strains to local and nonlocal collections under natural field conditions. In view of the demonstration of heterothallism and frequent mutation in *Ustilago zeae,* the constancy of reaction of strains of corn to natural infection by local and nonlocal collections of the organism the same year or in successive years is of extreme importance. Mutations have been shown to occur in monosporidial lines of the smut organism in surprisingly large numbers (1, 15, 16). These mutants can then hybridize and new combinations of characters can be produced (16), since *U. zeae* is heterothallic (14).

Jones (11) found resistance to smut infection to be dominant in the F_1 generation of crosses between selfed lines that differed in smut reaction. Hayes *et al.* (7) found F_1 crosses between resistant strains slightly more resistant than either parent, while F_1 crosses between resistant and susceptible strains were intermediate in reaction. Immer and Christensen (9) corroborated the conclusion reached in the earlier Minnesota studies that dominance of resistance or susceptibility was lacking in F_1 crosses between inbred lines differing in smut reaction. In later studies, Immer (8) again reported F_1 crosses to be intermediate in reaction, although several cases of apparent dominance of susceptibility were found. A slight tendency to approach the more susceptible parent was found in back-crosses and in F_3 populations.

The purpose of the present paper is to present further data on the determination of losses due to smut in corn, to compare the reaction of inbred lines to natural infection in epiphytotics from local and nonlocal smut collections, and to determine further the mode of inheritance of resistance or susceptibility to smut in crosses between inbred lines.

MATERIALS AND METHODS

The selfed lines and crosses of corn used in all parts of this study, except those dealing with losses due to smut infections, were grown in two systematically distributed plots of 30 to 40 plants each. A smut epiphytotic was induced in these plots and notes were taken on size and location of smut infections at two or three times during the growing season, as described previously (8, 9).

The study of losses due to smut infections in selfed lines was conducted as described previously (10), i.e., the yield of oven-dry, shelled corn from two plants growing one foot apart was compared, one plant bearing a single smut gall and the other plant of the pair being smut-free. These data were collected in selfed lines, inbred for five or more generations. Notes were taken on location of galls on the plants as well as size of galls.

The study of smut-induced losses in F_1 crosses of corn was made in the regular yield trials of these crosses. Two plants were selected from a hill of three, one plant being infected with a single gall and the other being free from smut galls. Notes were taken and the results analyzed in the manner described for selfed lines.

In 1928 and 1929 all strains of corn tested in the "smut plots" were grown in two replicated single-row plots in each of two widely separated fields. In field E a smut epiphytotic was created, using smut collected at University Farm, St. Paul, Minnesota, only. Part of the field had been used as the corn smut plot for each of 2 years prior to 1929 and the other part had been so used for the 8 years preceding 1929. Conditions in this plot were, therefore, very favorable for smut infection. In another field, X–28, separated from field E by a distance of more than 1 mile and by certain natural barriers to the spread of smut spores from one field to the other, the same strains were grown in an artificially induced epiphytotic of smut collected from numerous sources in the northern Mississippi Valley as well as at University Farm. Many of the forms of smut present in field E probably were present in X–28, as well. The purpose of this phase of the study was to compare the reaction of selfed lines and crosses of corn to natural infection by local collections of the smut organisms with their reaction to a combination of both local and wide smut collections.

EXPERIMENTAL RESULTS

Losses due to smut: The losses induced by smut galls located on the corn stalks is of interest to plant pathologists and plant breeders, alike. The results of the experiments to determine the losses due to single smut galls of different sizes and on different parts of the plants, apart from the ears, covering a period of 3 years in the case of selfed lines and 2 years for F_1 crosses, are given in table 1.

Losses due to smut galls appear to be directly correlated with the size of the galls. The data on damage due to large and small galls both above and below the ears indicate that single large galls (4 in. or more in diameter) reduce the yield of shelled corn on the infected plants approximately four times as much as small galls (less than 2 in. in diameter).

There seems little difference in damage induced by smut galls at the base or first shoot as compared with the losses from smut at higher shoots

TABLE 1.—*Loss in shelled corn due to single smut galls on plants of selfed lines or F_1 crosses. Data on selfed lines collected 1927–29 and F_1 crosses 1928–29*

Location of galls	Size[a]	Selfed Lines			F_1 Crosses		
		Number of comparisons	Per cent loss	Odds that loss is significant	Number of comparisons	Per cent loss	Odds that loss is significant
Base and first shoot	L	14	55	1499:1	3	26	14:1
" " " "	M	47	21	189:1	8	26	13:1
" " " "	S	35	12	42:1	6	15	6:1
Second and higher shoots	L	25	57	>10000:1	13	67	1833:1
" " " "	M	31	18	38:1	16	21	30:1
" " " "	S	23	18	44:1	9	5	3:1
Neck	L	21	99	>10000:1	21	58	>10000:1
"	M	27	45	1999:1	57	33	>10000:1
"	S	24	−4	2:1	49	15	294:1
"	All	72	45	>10000:1	127	31	>10000:1
Total infection below ear	L	43	61	>10000:1	17	54	>10000:1
" " " "	M	80	22	>10000:1	25	23	108:1
" " " "	S	61	15	1193:1	15	9	12:1
" " " "	All	184	29	>10000:1	57	31	>10000:1
" " above "	L	38	95	>10000:1	22	59	>10000:1
" " " "	M	40	41	>10000:1	58	33	>10000:1
" " " "	S	37	1	1:1	53	15	591:1
" " " "	All	115	47	>10000:1	133	31	>10000:1

[a] L = Large galls; *i.e.*, 4 in. in one or more diameters.
M = Medium galls; *i.e.*, larger than "small" and smaller than "large" galls.
S = Small galls; *i.e.*, less than 2 in. in any diameter.
All = All galls at a given location.

or ear buds on the plants of selfed lines. Jorgenson (12) found this same condition in Ohio. The data from F_1 crosses were too meager to draw any conclusions on this point.

Large or medium smut galls above the ears of selfed lines did greater damage than galls of similar size below the ears. Large galls on the necks of plants from inbred lines reduced yields by 99 per cent. Such large galls resulted in barren stalks in almost every instance. In F_1 crosses infections above the ears also did slightly greater damage than infections below the ears. The difference was not so great, however, as in selfed lines. Jorgenson (12) also found that galls above the ear in F_1 crosses caused greater losses than galls below the ears.

There seems to have been little difference in the reduction in yield in selfed lines and F_1 crosses due to smut galls below the ears. Large and medium galls above the ears were more destructive in selfed lines than in F_1 crosses. Damage is dependent, to a large extent, on the size of the galls. For this reason comparisons of losses due to smut galls on different parts of the plant must be made on the basis of galls of definite size. For example, in the data given in table 1, 30 per cent of the galls below the ear in F_1 crosses are seen to be large galls, while only 17 per cent of the galls above the ear were large. Obviously, the average infection of all galls at these two locations does not give a critical comparison of the losses because a greater percentage of large galls were involved in the first instance and large galls result in greater losses than do small ones. Therefore, analyses of damage caused by smut must be made on the basis of both size and location of infections.

An indication had been found previously (8) that very susceptible selfed lines tended to have a greater number of smut galls than resistant strains. A study was made of the inbred lines grown from 1924 to 1929, this time dealing with the size of galls on resistant and susceptible strains. Only plants with a single smut gall were considered in this study. In the selfed lines with less than 15 per cent of the plants smutted, 11 per cent of the infected plants had large galls, 43 per cent medium, and 46 per cent small galls. In the selfed lines with more than 50 per cent of the plants smutted, 25 per cent of the galls on the infected plants were large, 49 per cent medium, and 26 per cent small. It is evident, therefore, that susceptible strains of corn not only tend to have more smut galls per plant but these galls tend to be larger on infected plants in susceptible than in resistant strains.

Considering that the losses due to smut are directly associated with size of gall, the notes on percentage of plants infected by smut is a very conservative measure of the relative resistance of different strains. The difference in damage due to smut in resistant as compared with susceptible

strains is considerably greater than would be indicated by the note on percentage of plants infected.

From the data given in table 1 it is possible to develop a more accurate technique than has been heretofore available for estimating the losses caused by smut in making disease surveys. Losses from ear smut can be readily determined by estimating the percentage of ears destroyed. To this must be added the damage due to smut galls on other parts of the plants. On the basis of the data available this can be done by estimating the losses in shelled corn traceable to large galls on the infected plants at 50 per cent, by medium galls at 25 per cent, and small galls at 10 per cent. The actual losses (Table 1), are but slightly greater. If it is not convenient to classify the galls on the basis of size in making the disease survey it seems safe to estimate the losses, due to all galls on the stalks of infected plants, at 30 per cent. Estimates in which size of gall is considered will, however, be more accurate.

REACTION OF SELFED LINES TO LOCAL AND NONLOCAL COLLECTIONS OF SMUT

Since corn smut causes great losses in yield of grain it is not only of great interest to determine the constancy of reaction of resistant selfed lines to smut but to determine if such selfed lines will react in the same manner to local and nonlocal collections of the smut organism. In table 2 is given the smut reaction of 15 selfed lines to local smut collection over a period of 6 years. Each of these strains has been inbred for at least 8

TABLE 2.—*Smut reaction of selfed lines grown in a smut epiphytotic for 5 or 6 years*

1929 Culture No.	Variety	Years selfed	Percentage of smut						
			1924	1925	1926	1927	1928	1929	Ave.
S-1	Rustler	10	84	100	89	100	76	.....	90 ± 4
-4	Minn. No. 13	10	74	97	52	46	13	33	53 ± 4
-6	Longfellow	8	52	77	69	79	63	.....	68 ± 5
-7	"	10	27	27	17	46	18	21	26 ± 3
-8	"	10	15	9	10	13	2	6	9 ± 2
-9	King Philip	8	.....	100	78	100	76	99	91 ± 4
-10	Rustler	9	71	82	80	100	56	.....	78 ± 4
-11	"	11	12	20	16	23	13	22	18 ± 3
-12	"	9	13	10	17	22	23	21	18 ± 3
-13	"	10	10	4	10	5	4	0	6 ± 2
-14	"	9	.....	9	8	0	3	2	4 ± 2
-15	"	10	.....	9	2	7	3	0	4 ± 2
-20	Minn. No. 13	8	2	20	16	16	7	0	10 ± 2
-21	"	8	.....	0	3	0	0	0	1 ± 2
-31	N. W. Dent	8	4	9	5	9	1	4	5 ± 2

generations. The uniformity of reaction to local smut collections under field conditions is demonstrated clearly.

Selfed lines, differing greatly in resistance to smut, can be obtained in any of the varieties of corn used in breeding work at University Farm. These selfed lines usually breed true for a particular manner of reaction to smut, particularly after they have been selfed for a number of generations and approach homozygosity.

In 1928, 358 and in 1929, 281 selfed lines of corn were grown in two systematically replicated plots in each of two fields at University Farm. In field E, an artificial epiphytotic was created, using smut collected at University Farm, only. In field X–28 an artificial epiphytotic was created in which numerous smut collections from the northern Mississippi Valley,[3] including collections from field E, were used. Many of the smut forms present in field E probably were present in field X–28. The two fields were separated by a distance of more than 1 mile and by certain natural barriers.

A comparison was made of the reactions of these selfed lines to local collections of smut and to the local and nonlocal smut collections by means of correlation studies. If the correlation between the reaction in the two replicates on a single field is of essentially the same magnitude as the correlation between single replicates of the two different fields, it seems safe to conclude that the selfed lines reacted in essentially the same manner to the different smut collections. If, however, the correlation between smut reaction in the two fields was much less than between replicates on the same field, either the different physiologic forms of smut encountered affected the reaction of the selfed lines in a differential manner or cultural conditions in the two fields may have been sufficiently different to cause the seemingly differential response. The results of the study are given in table 3.

TABLE 3.—*Correlation between smut reaction of selfed lines grown in two replicates in two different fields; field E being inoculated with smut from University Farm and field X–28 with smut collected from many sources in the northern Mississippi Valley, including University Farm*

Nature of correlation	Correlation coefficient	
	1928	1929
First and second replicate in field E	+.60 ± .02	+.65 ± .02
" " " " " " X–28	+.60 ± .02	+.65 ± .02
First replicate in E with first replicate in X–28	+.36 ± .03	+.63 ± .02
Second replicate in E with second replicate in X–28	+.39 ± .03	+.68 ± .02
Average of both replicates in E with both replicates in X–28	+.47 ± .03	+.75 ± .02

[3] The writers wish to express their thanks to C. Anderson, R. Bulger, W. Butler, G. Curran, G. Mayouse, L. Person, A. Thiel, H. Thornberry, J. Wallace, and others for their kindness in supplying collections of the smut.

In 1928 the soil was very dry at planting time. Field E was planted first and the corn germinated fairly well. Field X 28 was planted 2 days later. As a consequence of this delay and the fact that field X–28 was not in so good a state of tilth at planting time, most of the seed in X–28 did not germinate until rain came, almost 2 weeks later. The delay in germination of the seed in field X–28 may account for the lower correlation between individual series of the two fields as compared with the correlation between series of the same field. It is known that the reaction of corn to smut is affected by the stage of development of the corn plants. The possibility of different physiologic forms of smut affecting the reaction of these corn strains and causing part or all of this difference is not, however, overlooked.

In 1929 soil conditions at planting time were more favorable. Both fields were planted within a period of 2 days and developed in a very comparable manner. The results obtained in 1929 appear to be more truly representative of the similarity or difference in response of these corn strains to nonlocal collections of smut. The fact that the correlation between individual replicates of the two different fields is essentially the same as the correlation between the two replicates on the same field indicates clearly that these selfed lines of corn reacted in the same manner to the two smut collections in 1929. In examining the correlation tables, showing the relationship between reaction in fields E and X–28 for both 1928 and 1929, it was noted that the reactions of a few lines, to the different smut collections, were somewhat at variance with those of the bulk of the selfed lines, as indicated by their rather wide departure from the regression line. The regression line showed the general trend between reaction of these strains in the two fields in each of the 2 years. In the case of each strain departing widely from the general trend between the two fields and grown both years, it was found that this wide departure from the regression line held for only 1 year, indicating that such departures from the average reaction were due to chance fluctuation and were not inherited from year to year. This furnishes additional evidence that the corn strains grown in 1929 reacted in essentially the same manner to smut collections made at University Farm and to collections of smut from a wider source when tested for reaction to natural infection in the field and subject to an epiphytotic of the disease.

It should, however, be emphasized that these conclusions are based on the general reaction of a large number of selfed lines to the two different smut collections. As only 30 to 40 plants were grown per replicate it was not possible to determine whether some few lines did react in a differential manner. The evidence available from this study indicated that, if there was a differential response by a few selfed lines to the two different smut

collections, this differential reaction was not very great. It should be remembered, however, that these conclusions are based on 2 years' results and that further tests are in progress. If similar results are obtained from other studies, it will greatly facilitate the production of smut-resistant corn.

REACTION OF F_1 CROSSES

Since the immediate solution of the corn-breeding problems probably will be the production and distribution of desirable hybrids between selfed lines, the reaction of F_1 crosses, as compared with that of the parental inbred lines, is of considerable importance. Eighteen such crosses between inbred lines of known reaction to smut were made in 1928. These crosses were grown in 1929 and their reaction compared with that of the parent cultures. Selfed lines with an average of 0 to 15 per cent of smutted plants were designated as low-smut lines, those with 15 to 50 per cent of smutted plants were called medium-smut lines and lines with more than 50 per cent of smutted plants were designated high-smut lines. The results are given in table 4.

In the 8 crosses between inbred lines differing in smut reaction, i.e., crosses of high x low or medium x low smut lines, 4 crosses were more resistant and 4 more susceptible than the average of the parents. This difference, however, was not appreciable, considering the magnitude of the probable errors. In the 10 F_1 crosses between low-smut lines, 9 were slightly more susceptible and 1 slightly more resistant than the average of the parent inbred lines. The greater susceptibility of the F_1 as compared with the average of the parents in low x low-smut crosses may be due to different genetic factors determining smut resistance in the different parents or to the fact that the more vigorous plants in the F_1 crosses may be slightly more susceptible than the smaller plants in the inbred lines. This latter possibility was tested by calculating the correlation between smut reaction and height of plants in 300 F_3 lines from a composite cross of 8 inbred lines. A significant correlation was not found.

Combining this group of F_1 crosses with those reported previously from Minnesota, 34 F_1 crosses between inbred lines of known smut reaction have been tested. The general conclusion to be drawn is that the F_1 tends to be intermediate in reaction.

COMPOSITE CROSS OF EIGHT SELFED LINES

In 1925, a composite cross was made of 8 supposedly low-smut lines. One of these proved to be medium in reaction in subsequent tests but the other 7 have remained quite resistant. The recombination was made by pollinating each line in turn with a mixture of pollen from the other 7. The reaction of the F_1 has been reported previously (8). A number of F_1

TABLE 4.—*Reaction of parent selfed lines and F_1 crosses of corn to smut infection*

1928 Cross	Variety	Per cent smut in parent selfed lines								Type of cross	Per cent smut in in F_1
		First parent				Second parent					
		1926	1927	1928	1929	1926	1927	1928	1929		1929
1600 × 300		64	100	92	90	12	16	6	13	High × Low	62 ± 7
1600 × 1700		64	100	92	90	.....	21	4	4	" × "	35 ± 11
600 × 1500		76	83	38	.....	30	14	8	8	" × "	52 ± 11
1200 × 100		71	75	32	95	16	14	0	2	" × "	27 ± 9
1100 × S–3		70	86	39	71	0	.....	6	.....	" × "	24 ± 9
400 × S–3		49	.....	27	.....	0	.....	6	.....	Med. × "	9 ± 4
S– 3 × S–15	Rustler	0	.....	6	.....	8	0	3	2	Low × "	18 ± 7
S– 3 × S–16	"	0	.....	6	.....	2	7	3	0	" × "	6 ± 4
S–14 × S–15	"	10	5	4	0	8	0	3	2	" × "	16 ± 7
S–14 × S–16	"	10	5	4	0	2	7	3	0	" × "	6 ± 4
S–15 × S–16	"	8	0	3	2	2	7	3	0	" × "	18 ± 7
S–C × S–8	Longfellow	13	.....	39	38	10	13	2	6	Med. × "	21 ± 9
S–23 × S–28	Minn. No. 13 ...	3	0	0	0	27	0	35	2	Low × "	18 ± 7
S–25 × S–28	" ...	0	0	0	2	27	0	35	2	" × "	24 ± 9
S–B × S–A	N. W. Dent	.....	19	10	20	5	9	1	4	Med. × "	37 ± 11
S–F × S–G	Silver King	9	4	14	19	2	11	12	9	Low × "	12 ± 7
S–F × S–H	" "	9	4	14	19	6	10	4	.....	" × "	19 ± 7
S–G × S–H	" "	2	11	12	9	6	10	4	.....	" × "	11 ± 7

TABLE 5.—*Smut reaction of eight parent lines and F_1, F_2, F_3, and F_4 generations after a combination of these selfed lines*

	Year	Frequency distribution of lines with regard to per cent smut						No. lines
		0.0–10.0	10.1–20.0	20.1–30.0	30.1–40.0	40.1–50.0	50.1–60	
Parent lines	1926	4	3	.....	1	.....	.....	8
F_1 "	"	3	3	1	1	.....	.....	8
Parent lines	1927	2[a]	2	2	.....	.....	1	7
F_2 "	"	1	6	12	9	3	3	34
Parent lines	1928	5	2	1	.....	.....	.....	8
F_3 "	"	109	112	40	23	8	7	299
Parent lines	1929	4[b]	.....	2	.....	.....	.....	6
Sel. F_4 "	"	52	45	26	10[b]	2	1	136

[a] No test of one parent line in 1927. Should have fallen in this class on basis of past performance.

[b] No test of two parent lines in 1929. Should have fallen in these two classes on basis of past performance.

ears were selfed and 34 F_2 lines were grown. A large number of F_2 plants were then selected at random and selfed in order to provide a large, random F_3 population. The most desirable F_3 lines, as judged from notes on plant and ear characters as well as smut resistance, were carried into F_4 by selfing. The F_4 lines, were, therefore, selected material. The smut reaction of the F_1, F_2, F_3, and selected F_4 lines was determined in a smut epiphytotic in relation to the reaction of the parent cultures. The results are given in table 5.

The F_1 recombination lines reacted essentially as would be expected on the basis of previous studies with F_1 crosses, i.e., like the average of the parents. The F_2 lines were somewhat more susceptible, in general, than the F_1. In 19 of the 34 F_2 lines less than 30 per cent of the plants were smutted, and in the other 15 lines more than 30 per cent were infected, while only 1 of the 8 parent lines had more than 30 per cent smutted plants.

The reaction of the 299 F_3 lines, grown from selfed seed of a large number of F_2 plants selected at random, is of greatest interest. Seventy-four per cent of the F_3 lines had less than 20 per cent, 87 per cent had less than 30 per cent smutted plants, and 95 per cent less than 40 per cent. It seems, therefore, that these F_3 lines reacted very nearly the same as the average of the parents. One parent was known to be a medium-smut line. The probable error of the reaction of a "30 per cent smut" line was about 10 per cent. Chance fluctuation could, therefore, account for the reaction of some of the apparently susceptible F_3 lines. In the main the same genetic factors seem to have controlled resistance to smut in those 8 parent selfed lines. The most susceptible F_3 lines were discarded in 1928 and only the more resistant lines continued in 1929. Only 3, or 2 per cent, of the F_4 lines had more smut than any of the parent inbred lines. The smut epiphytotic of 1929 was somewhat more severe than that of 1928 but the effect of the elimination of the most susceptible F_3 lines is clearly indicated. Apparently, lines can be produced very easily from recombinations of low-smut strains which are as smut resistant as the parent cultures.

SUMMARY

1. A study was made to determine the losses caused by smut infection in F_1 crosses and in selfed lines of corn inbred five or more generations.

2. Size and location of smut galls on the plant were important. The larger the galls on the stalks, the greater was the reduction in yield of shelled corn. Large or medium-size smut galls on the stalk above the ears did greater damage than galls of similar size below the ears.

3. Susceptible lines of corn tend to have a greater number of smut galls per plant and larger galls than resistant lines.

4. From the available data it was estimated that the reduction in yield in shelled corn resulting from large, medium, and small galls on stalks was 50 per cent, 25 per cent, and 10 per cent, respectively.

5. Selfed lines that differ greatly in resistance to smut can be obtained in all varieties of corn used in breeding work at University Farm.

6. Fifteen lines of corn inbred for at least 8 years reacted in a very similar manner for each of 6 different years when grown in an epiphytotic of smut at the University Farm.

7. In 1928 and 1929 the extent of infection of numerous lines grown in the field in which smut collections from University Farm only were used was correlated with the infection obtained in a different field where smut collections were from a wider source. The calculated correlation coefficients were $.47 \pm .03$ and $.75 \pm .02$, respectively.

8. The smut infection of 34 F_1 crosses was compared with that of the parents. Crosses between two resistant lines were fairly resistant, although they were more severely infected than the parents.

9. A composite cross was made of seven low-smut lines and of one medium-smut line. From a study of 299 F_3 lines selected at random 87 per cent were no more susceptible than the most susceptible parent. The results indicate that composite crosses will undoubtedly yield smut-resistant lines in a large percentage of cases.

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THE RELATION BETWEEN INSECT AND VIRUS AS SHOWN IN POTATO LEAF ROLL, AND A CLASSIFICATION OF VIROSES BASED ON THIS RELATION

D. L. Elze

Soon after Oortwijn Botjes, Schultz, *et al.* had realized the importance of aphids in spreading potato leaf roll and mosaic, they began to investigate the part played by other insects in the spread of these diseases. These investigations, however, led to no positive results (16, p. 55; 19, p. 329; 21, p. 50).

In 1922 Murphy (13) believed that he had succeeded in proving that leaf hoppers, capsid bugs, and probably the potato flea beetle are able to transmit leaf roll and from field observations deduced that these insects play an even more important part than aphids, the latter being less numerous. His research method, however, was such that the results cannot be considered as altogether trustworthy. He used for each of the above-mentioned species only one experimental plant and, during their development, the plants were not inspected for the posibility of other infections. With later experiments he and others obtained widely different results. Moreover, Elze (5) proved by experiment and field observation that the significance of aphids, especially of *Myzus persicae* Sulz., should not be sought so much in their large increase on the potato during the summer as in the abundance of the first alate generations which in early summer pass from other plants on to the potato. These winged generations are, however, rarely so numerous as to be conspicuous with field observations.

In 1927 Smith (25) published his first investigations on the significance of various sucking insects in the spreading of mosaic. He made use of some species of aphids, white fly, leaf hoppers, and capsid bugs, and found that in the given order the transmitting power decreased. He tried to explain this from the regularity with which these insects tap the phloem with their stylets. On this subject he published a separate article in 1926 (24). In this paper he starts with the supposition that all these insects transmit the virus purely mechanically but does not mention the grounds on which this supposition is based. Observations on virus diseases of various other plants do not lend support to this theory.

In later researches on the spreading of leaf roll and mosaic by the principal insects mentioned above and also by the potato flea beetle Smith (26, 27) succeeded in getting positive results only with *Myzus persicae*. His conclusion that other potato-virus workers apparently succeed more easily in transmitting these diseases with various insects would not appear ex-

actly correct. In their experiments with various insects Schultz and Folsom (18, 19, 20, 21, 22) have been able to transmit these diseases exclusively by aphids, in which case they have, in general, worked with a large number of plants and insects. In their last paper (8) they mention the probability that also other insects may play an important part, but this is not based on exact experiments. That Elze (5) succeeded in transmitting virus diseases by nearly all insects he used, both sucking and others, should also be ascribed in large part to the fact that he infected, in general, a greater number of plants than did Smith. Moreover, he kept the plants growing for a longer period owing to the circumstances under which the experiments were performed, so that the chance of the virus reaching the tubers grew considerably. In several cases also Elze reinfected the plants with virus-bearing insects in the course of the same experiment. To this ensemble of factors may be ascribed his success in transmitting leaf roll and mosaic by means of leaf hoppers, capsid bugs, flea beetles, and caterpillars of *Mamestra brassicae* L. On account of these experiments and his field observations, however, he concludes that all these insects are of hardly any significance for the spreading of virus diseases under normal circumstances. Among the aphids, *Myzus persicae* is by far the most important. Of the other aphids, *Aphis rhamni* B. de Fonsc. (*abbreviata* Patch, see Elze 5, p. 32) and *M. pseudosolani* Theob. transmitted various diseases; with *A. fabae* Scop. only mosaic was transmitted in a few cases.

The particular significance of *Myzus persicae* is explained by Elze from the fact that a definite biological relation between the virus (in this case of leaf roll) and *M. persicae* seems to exist. This is apparent from:

1. The existence of an incubation of the virus in the insect.
2. The retention of the infecting power of the insect after the molt.
3. The preservation of this power after the aphids have lived for about a week on plants not susceptible to leaf roll (spinach).
4. The great certainty with which a small number of individuals can transmit the disease.

These observations were confirmed in 1929 by Smith (26), as was the fact that the virus is not transmitted from the mother insect to the larva.

Lastly, Murphy and McKay (14) published in 1929 the results of their researches, carried on since 1922, on the dissemination of leaf roll by various insects, more especially by aphids. They fully confirm the fact already stated by other investigators that, besides *Myzus persicae*, other insects are of little significance. This fact was doubted by them in former publications. Yet it is remarkable that also with *Macrosiphum solanifolii* Ashm. and *Myzus pseudosolani* so few results were obtained. Especially on *Macrosiphum solanifolii* this is not in accordance with the facts recorded by Schultz and Folsom (19, 20), though Smith (26, 27) also got no positive results from his few experiments.

In order to study the differences of disease-disseminating power of various insects in a more exact way, the following experiment was carried out by the writer in 1928. The method described earlier (5, p. 38) was used. In each of 4 separate but adjoining greenhouses, 22 healthy tubers were planted together with 1 tuber from a leaf-roll plant. The 22 healthy tubers in each greenhouse consisted of 8 tubers of the variety Paul Krüger (President), 7 of Eersteling (Duke of York), and 7 of Roode Star (Red Star). All tubers were planted in separate pots; otherwise, there was no partition between the plants in any one house. In this way repeated infection of the healthy plants by insects from the diseased plant was possible. A path divided each greenhouse into 2 parts. The diseased tuber in each case was cut in half, the halves were planted in separate pots, and the pots were placed one on each side of the path. The healthy plants of the 3 varieties were placed around the diseased one in a regular manner, so that the chance of infection might be equal for each variety. Conditions were essentially the same in each of the 4 greenhouses. For the spreading of the disease in the first house, *Myzus persicae,* in the second, *Aphis rhamni,* in the third, *A. fabae,* and in the fourth, the flea beetle, *Psylliodes affinis* Payk., were used. The strain of *M. persicae* had for some years been raised in the greenhouses on healthy potatoes and on spinach, and that of *A. rhamni* on healthy potatoes and on *Rhamnus cathartica* L. The potatoes on which these strains had been raised during 1928 were still healthy in 1929. *Aphis fabae* was gathered in the field from broadbeans and occasionally from healthy potato plants standing among the beans. In the neighborhood no diseased potatoes were to be found. Notwithstanding these precautions, there was perhaps some danger of the transmission of diseases by *A. fabae* from the field into the houses. This method, however, was adopted because in the environment of Wageningen there is no extensive spread of *A. fabae* to potatoes and multiplication on this host is usually slow. It was expected that by choosing insects which already had passed on to potato and had multiplied there sufficiently, this difficulty would be presented in less degree. The flea beetle, *Psylliodes affinis,* had to be gathered on potato exclusively; this was done on a part of the experimental plots where only healthy potatoes were growing.

As some diseased (leaf roll) tubers of the Green Mountain, Irish Cobbler, and Rural varieties from America were available, these varieties were used in the experiment. This was done in order to determine whether net necrosis, observed in America as a symptom of primary leaf roll, can be produced in Dutch varieties when leaf roll from America serves as a source of infection. For comparison with the leaf roll of American source a diseased tuber of the Dutch variety Bevelander was used. The experiment was arranged so that in the house where *Myzus persicae* was the

vector, the Green Mountain variety was used as a source of disease; in the house where *Aphis rhamni* was the vector the Bevelander was used; in the house with *A. fabae* the Rural was used; and in the house with *Psylliodes affinis* the Irish Cobbler was used.

The results of this experiment as to the appearance of net necrosis are published elsewhere (6). Such a combination of the solution of two problems in one experiment was considered admissible because, from a comparison of the American and the European literature on leaf roll, it probably is safe to conclude that leaf roll is identical on both continents, a fact confirmed by these results.

The results as to the spreading of leaf roll are presented in table 1.

TABLE 1.—*Rate of spread of potato leaf roll in the presence of various insects*

House	Insect	Source of infection	Infested with leaf roll			Total
			Pres.	D. o. York	Red Star	
1	*Myzus persicae*	Gr. Mountain	8	7	7	22
2	*Aphis rhamni*	Bevelander	4	4	5	13
3	*A. fabae*	Rural	3	1	2	6
4	*Psylliodes affinis*	Irish Cobbler	3	2	3	8

Before entering into a more detailed discussion of these results the following comments should be made: The next year's crop of the plants from the first house all showed, besides leaf roll, a disease of the mosaic type; apparently the Green Mountain tubers used had, besides leaf roll, a mosaic disease. Likewise, several plants from the houses with Rural and Irish Cobbler, respectively, showed the next year, besides leaf roll, thin weakened stalks that were strongly suggestive of spindling sprouts. Before the planting, however, the sprouts were quite normal. Though the plants concerned were those on which insects, caught in the field, had been put, it is very improbable that the cause of this malady should have been transmitted by these insects, as such a disease was only sporadically present in the experimental field plots from which they were taken. The disease factor must have been transmitted thus from the leaf-roll plants of the Rural and Irish Cobbler varieties. As it did not appear in either of the two other greenhouses it cannot be a symptom of leaf roll, as Schultz and Folsom (20) originally thought spindling sprout to be. One rather gets the impression of a separate virus disease, as had been surmised before by Elze (5, p. 45).

The tubers of the Duke of York variety were of the same origin as those with which Quanjer and Oortwijn Botjes (17) produced top necrosis by grafting on the variety President. Though with these experiments both varieties were used side by side and there was nothing to interfere with the passage of the insects from a Duke of York plant to a President, the next year's crop of President showed in no case top necrosis. An explanation of this is difficult; possibly the insect did not take the virus of top necrosis so easily, as most of them were infected already with the leaf-roll virus. Possibly the top necrosis is like aucuba mosaic, difficultly transmissible by insects. The last supposition is made probable by the fact that the Duke of York variety is regularly cultivated by the side of other varieties without the occurrence of top necrosis on the latter.

Returning to the discussion of the results concerning the problem why the experiment was started, it appears that in all cases a rather high percentage of the plants had become diseased. This seems to be in contradiction to the English-Irish experiments, so far as it concerns the plants on which *Aphis rhamni, A. fabae,* and *Psylliodes affinis* were brought; this contradiction, however, is not so important as it would seem to be. The circumstances under which the plants were grown in the houses, viz, comparatively high temperature and humidity and scarcity of light, lengthened their growing period. Thus, the chance of infection increased, as well as the possibility that, after the infection, the virus was able to reach the tubers. Already, in the description of the arrangement of the experiment, the possibility of a repeated infestation of the healthy plants was advanced. In the houses with *Myzus persicae* and *Aphis rhamni* these aphids multiplied so fast that, to prevent an early withering of the plants, these houses had to be fumigated with a nicotine preparation. After the fumigation new aphids were brought on to the plants. *Aphis fabae* multiplied less strongly. To contrive, however, a regular dissemination over all the plants and to be sure of a repeated infestation, several aphids were at intervals transmitted from the diseased to the healthy plants. With *Psylliodes affinis* this was more difficult, yet the regular spreading of this insect was managed. These beetles enter the soil for propagation and soon die there. For some months, therefore, beetles just caught were placed 2 or 3 times per week on the diseased plants. The following day the insects still feeding on them were spread over the healthy plants. In this way the possibility of a maximum infection was created in all cases. In spite of this, except in the house with *M. persicae,* a smaller or larger number of plants remained healthy. This difference in attack can be explained only from a real difference in the relation existing between the virus and the various insects used.

In attempting to explain the difficulty with which leaf roll is transmitted by the potato flea beetle, it must be remembered that in insects with

cutting mouth parts the salivary canal usually ends in the pharynx, in which organ the food is mixed with the saliva. With the sucking insects, on the contrary, the sucking canal and the salivary canal are separated from each other as far as the end of the setae. Thus, saliva is first injected into the living plant cell, after which the cell contents are withdrawn by the insect through its sucking canal. With the exception of the probably rare cases of accidental infection through virus sticking to the mouth parts, it must be assumed that with sucking insects the infection of healthy plants takes place through the saliva. The virus is sucked in together with the food and diffuses from the intestinal canal into the blood and from there into the salivary glands to arrive with the saliva back into the plant. It appears from numerous well-studied cases that the infection by sucking insects really takes place in this way. In the first place, the infection occurs with greater certainty than can be explained from the infection with virus residue sticking to the mouth parts. Secondly, it has been shown that the virus is not lost with molting and is preserved if the insects live for some time on plants not susceptible to the disease. See Severin (23), Carsner (2), and others for curly top of sugar beet; Kunkel (11) for aster yellows; McClintock and Smith (12) for spinach blight; Storey (28) for streak of maize; Elze (5) for potato leaf roll; and Ogilvie (15) for a virus disease of lily.

It can be assumed that with insects with cutting mouth parts the virus taken with the food diffuses likewise from the intestinal canal through the blood to the salivary glands. This being true, the virus will come back into the plant very rarely with the taking of food, as the food is not mixed with a virus-bearing saliva before it comes in the pharynx. Consequently, it may be assumed that infection by the potato flea beetle will take place only accidentally through virus residue sticking to the mouth parts.

More difficult is the explanation of the differences in transmitting power of the three species of aphids used. The feeding habits of aphids have been studied by several investigators, and over and over again the observations of Büsgen (1) and Zweigelt (30) have been confirmed, that these insects penetrate with the stylets into the phloem; for *Myzus persicae*, among others, by Smith (24) and for *Aphis fabae* by Davidson (3), while the writer has observed the same for *A. rhamni*. Thus the differences in transmitting power cannot be explained as a mechanical phenomenon. The most plausible concept is undoubtedly the assumption that with the various aphids the leaf-roll virus is attacked to a different degree by the internal organs and their secretions, either in the intestinal duct, in the blood, or in the salivary glands. In other words, there exists a more or less pronounced adaptability of the virus to the various aphids. With *M. persicae* this adaptability is most marked. The researches of Elze (5)

and Smith (27) point to the fact that the virus is able to keep alive for some time in this insect and probably to multiply itself. With *A. rhamni* this adaptability is much weaker, whereas with *A. fabae* the virus seems to be destroyed entirely. Infection seems to take place here exclusively through virus residue that is accidentally present on the stylets. The results of Elze (5) and Murphy and McKay (14) with *M. pseudosolani* and of Schultz and Folsom (20, 21) and Murphy and McKay (14) with *Macrosiphum solanifolii* point to the probability that these aphids, as regards their adaptability are intermediate between *A. rhamni* and *A. fabae*.

If this theory about the connection between the leaf-roll virus and the different potato aphids is right, the following may be concluded:

First. Of each aphid species a certain percentage of the individuals that have lived on diseased potatoes will be able to transmit leaf roll after molting, without sucking any more on the diseased plants.

Second. This percentage of disease transmission by molted aphids will differ only slightly from that by nonmolted ones of the same species. Thus, this percentage will vary from nearly 0 with *Aphis fabae* to about 100 with *Myzus persicae*.

In order to study the correctness of these conclusions, the following experiments were made during the winter of 1929–30. Aphids of the species *Myzus persicae* and *Macrosiphum solanifolii* were reared on leaf-roll tubers. *Aphis rhamni* could not be made use of, for, until now, I have not succeeded in breeding this aphid parthogenetically during the winter, whereas *Aphis fabae* and *Myzus pseudosolani* are growing too poorly on sprouting tubers to make use of them for these experiments.

To be sure that after molting the aphids would not feed any more on the diseased tubers, nearly full-grown larvae were brought from the diseased tubers in little glass tubes and kept therein for a night. In each tube a single individual was kept and its molting observed. The next day the molted and nonmolted aphids were placed each on a separate tuber. The results are shown in table 2.

TABLE 2.—*Retention of the virus of potato leaf roll by aphids after molting*

	Transmission by nonmolted aphids		Transmission by molted aphids	
	Number of tubers		Number of tubers	
	Treated	Diseased	Treated	Diseased
Myzus persicae	11	10	10	7
Macrosiphum solanifolii	34	7	25	9

The number of treated tubers is rather small; still, from the figures, it is evident that *Macrosiphum solanifolii* does not lose the virus during the process of molting, just as has been shown earlier for *Myzus persicae* (5). However, the percentage of the treated tubers that became diseased was much smaller with *Macrosiphum solanifolii* than with *Myzus persicae,* and this was the case when molted as well as when nonmolted aphids were used. So, the results are in accordance with the assumption as to the cause of the differences of the potato aphids in transmitting leaf roll. However, contradictory to the results of Murphy and McKay (14) and Smith (27), the transmitting power of *Macrosiphum solanifolii* seems to be large enough to be significant whenever this aphid is numerous.

According to the researches of Elze (5) and of Smith (25, 26, 27), other sucking insects, such as leaf hoppers and capsid bugs, have no greater significance for the spreading of leaf roll than *Psylliodes affinis.* It is possible, as Smith (24) has shown, that this may partly be ascribed to the fact that these insects, while feeding, do not tap the phloem so regularly as do aphids. Relative to researches on such diseases as curly top, aster yellows, etc., where leaf hoppers are the only spreaders of the diseases, it does not seem probable that this circumstance is very important. It is rather to be assumed also that with these insects the virus, after reaching the intestinal duct, is for the greater part destroyed. It should also be taken into account that in many cases the plant cells react so violently to the sucking of these insects that the protoplasm soon dies. For the further spreading of the strongly parasitic virus through the plant this should be an unfavorable circumstance, as already Smith (24) has asserted.

In relation to this it should be noted here that, according to the description of Wille (29), the capsid bug *Piesma quadrata* Fieb., causing the "Kräuselkrankheit" of the beet in Germany, does not cause such a violent reaction as appears from the early symptoms of this disease. Wille states that the Kräuselkrankheit should be included among the virus diseases. Also, the leaf hoppers *Eutettix tenellus* Bak., *Cicadula sexnotata* Fall., and *Balclutha mbila* Naude apparently do not cause necrotic spots.

In America necrotic spots develop to a high degree through *Empoasca mali* Le Baron living on numerous plants, but, as far as known, this insect plays no part as a spreader of a virus disease. At least, nothing indicates that the spots observed by Eyer (7) and Granovsky (9, 10) are related to virus diseases.

Elze (5) has given a classification of the virus diseases based on the relation between the virus and the insect with each of these diseases. From subsequent publications it appears that nearly always, when studied more thoroughly, a very close biological relation exists between the virus and at

least one of the insects able to transmit it. This points strongly to the living nature of the virus.

The only exception hitherto known is cucumber mosaic, where, according to Doolittle and Walker (4), *Aphis gossipii* Glov., as well as the beetles *Diabrotica vittata* Fabr. and *D. duodecimpunctata* Oliv., loses its transmitting power soon after leaving the diseased plant, so that in this case infection takes place only mechanically. In the remaining cases a distinction can be made between diseases (such as curly top and aster yellows) that can be transmitted by but one insect species and diseases that can be transmitted by a greater number of insects. In the latter only one or a few of these insects stand in closer biological relation to the virus. The remaining insects transmit the disease only mechanically and are mostly of little importance. Spinach blight and leaf roll can be placed in this group. It may prove necessary to separate aster yellows from those diseases that are transmitted by one insect species because of the long incubation period (10 days) of the virus in the insect. In all other cases, where the incubation period is determined, it lasts only 1 or 2 days.

There yet remains a group of diseases in which the transmission by insects has not yet been successful. Among such are peach yellows, sandal spike, and abutilon mosaic.

SUMMARY

The present-day knowledge of the significance of various insects in relation to the spread of the virus diseases of the potato is discussed. *Myzus persicae,* which is particularly well adapted for spreading such diseases, has been studied especially in connection with leaf roll.

There are different opinions as to the ability of other insects to transmit the viruses of the potato. It is suggested that such differences are due to the use of different experimental methods. European research workers generally are of opinion that *Myzus persicae* is the only insect that plays an important part.

Experiments are described on the spread of leaf roll from the varieties Bevelander, Green Mountain, Rural, and Irish Cobbler to the varieties President, Duke of York, and Red Star by the aphids, *Aphis rhamni, Myzus persicae,* and *A. fabae,* and the flea beetle, *Psylliodes affinis.* In these experiments the most favorable circumstances for infection were provided. The table of results shows that *M. persicae* infected all 22 plants, *A. rhamni* 13, *A. fabae* 6, and *P. affinis* 8 plants.

The small transmitting power of *Psylliodes affinis* is ascribed to the fact that insects with cutting mouth parts can cause infection only through virus residue that may accidentally adhere to the mouth parts. With the aphids and other Hemiptera, on the contrary, the differences are explained

by the assumption that the virus taken with the food is more or less destroyed by the organs of the insect, according to the degree of adaptability of the virus to the insect.

The correctness of this assumption is made probable by an experiment, wherein it is shown that *Myzus persicae* and *Macrosiphum solanifolii* do not lose their transmitting power when molting and that the percentage of virus-bearing individuals that are able to transmit the disease is nearly the same before and after the molt. Under the same conditions the transmitting power of *Myzus persicae* was much greater than that of *Macrosiphum solanifolii* (Table 2).

Considering these results and those of other investigators, the following classification is given, based on the relation of virus diseases to insects.

1. Viroses which, according to our present knowledge, are not spread by insects, e.g., peach yellows, sandal spike.
2. Viroses which are spread by different insects and are not specially adapted to any of these insects, e.g., cucumber mosaic.
3. Viroses which are adapted to particular insects.
   - A. Which, in addition, are transmitted mechanically by other insects, e.g., potato leaf roll, spinach blight.
   - B. Which are transmitted only by the insect to which the disease is adapted.
     - a. With a short incubation period of the virus in the insect, e.g., curly top, streak of maize.
     - b. With an incubation period of about 10 to 14 days, e. g., aster yellows.

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EN AARDAPPELONDERZOEK,
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THE RELATION OF PHYSIOLOGIC SPECIALIZATION IN TILLETIA TO RECENT EPIPHYTOTICS OF BUNT IN DURUM AND MARQUIS WHEATS[1]

C. S. HOLTON[2]

INTRODUCTION

In a previous publication[3] the writer pointed out that, prior to 1925, bunt caused virtually no damage to durum wheats in the hard-red-spring-wheat region of the United States but that its prevalence and destructiveness have increased so greatly since that time that the disease has assumed major importance. Evidence was presented that this increase in the severity of bunt in durum was due to the existence of one physiologic form, or possibly several, of *Tilletia tritici* (Bjerk.) Wint., apparently not present or, at least, not widely distributed previously. The present paper reports the results of experiments that substantiate this conclusion. In addition, it has been found that Marquis, previously highly resistant in the field in the spring-wheat region, became severely smutted in many fields in 1930. The results of inoculations indicate clearly that this increased amount of bunt in Marquis apparently is due also to the existence of at least one hitherto unknown form of *T. levis* Kühn. Marquillo, a newly developed stem-rust-resistant common wheat also seems susceptible to the new form. Gaines[4] has reported similar experiences with certain bunt-resistant varieties of wheat in Washington.

MATERIALS AND METHODS

Ten varieties of common and durum wheats and of emmer were inoculated with four collections of *Tilletia tritici* and two collections of *T. levis* in the field at University Farm, St. Paul, Minnesota, in 1930 (Table 1). The seed was treated with formaldehyde (dip method), washed in water, and, when dry, inoculated at the rate of 0.5 gram of spores to 100 grams of seed. After inoculation, the seed was sown in triplicated, systematically-distributed, 8-foot rows in the field.

[1] Published with the approval of the Director of the Minnesota Agricultural Experiment Station as paper No. 978 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writer is greatly indebted to Dr. E. C. Stakman for invaluable suggestions offered in the writing of this paper.

[3] Holton, C. S. A probable explanation of recent epidemics of bunt in durum wheat. Phytopath. 20: 353–357. 1930.

[4] Gaines, E. F. New physiologic forms of *Tilletia levis* and *Tilletia tritici*. Phytopath. 18: 579–588. 1928.

TABLE 1.—*The percentages of smutted heads in ten varieties of wheat and emmer inoculated artificially with four collections of Tilletia tritici and two collections of T. levis at University Farm, St. Paul, Minnesota, in 1930*

Class, variety and C. I. number	Inoculum, source of inoculum, and percentage of smutted heads					
	Tilletia tritici				Tilletia levis	
	California	Manitoba, Canada	Devils Lake, North Dakota	Litchfield, Minnesota	Ivanhoe, Minnesota	St. Paul, Minnesota
Common						
Kota 5878	50.8	39.3	36.1	16.1	67.8	61.0
Ceres 6900	20.0	25.6	17.8	9.6	48.1	42.5
Preston 3081	3.1	4.0	3.3	0.3	8.8	5.8
Marquis 3641	2.8	3.6	0.0	0.0	23.3	9.0
Marquillo 6887	2.1	4.5	0.6	0.0	18.5	7.8
Ruby 6047	0.5	1.3	0.0	0.0	2.0	2.5
Hope 8178	0.5	0.0	0.0	0.0	0.0	0.0
Durum						
Mindum 5296	0.3	0.1	30.2	0.7	4.2	2.2
Pentad 3322	3.3	2.6	40.1	2.0	6.3	4.6
Emmer						
Vernal 3686	2.6	0.0	12.3	16.1	4.0	1.8

The percentages of infection (Table 1) are based on counts of 600 heads in all varieties except Mindum, in which case the percentages are based on the total number of heads, ranging from 449 to 600, in 3 8-foot rows. Except for Kota and Ceres, in which smutted heads were easily detected by external examination, smutted heads were determined by clipping each head three times. No distinction was made between partially and completely smutted heads.

A number of commercial wheat fields[5] in various sections (south, west, north) in Minnesota were examined for bunt infection. The percentages of bunt were computed from counts of bunted heads in 10 separate yard lengths of the drill rows, selected at random. Random samples were examined microscopically to determine the relative prevalence of the two species of bunt in the various fields. (Table 2).

RESULTS

The results obtained in the inoculation tests with four collections of *Tilletia tritici* confirm those which have been published—Holton, *l. c.* The California and Manitoba collections probably consist of identical forms, for the virulence of one was very similar to that of the other on the varieties tested (Table 1). Both collections were extremely virulent for Kota, the California collection producing 50.8 per cent and the Manitoba collection 39.3 per cent. In 1929 these collections produced 53.8 and 42.6 per cent of bunt, respectively, on Kota. All other varieties tested, except Ceres, appear to be highly resistant to these collections. The North Dakota collection was most virulent for the durum wheats, Mindum and Pentad, producing in these varieties 30.2 and 40.1 per cent of bunt, respectively, in 1930, as compared with 50.6 and 40.1 per cent, respectively, in 1929. All other varieties tested, except Kota, have been somewhat resistant to this form. The results obtained from this collection in 1930 established the validity of previous conclusions that the apparently increased susceptibility of durum wheats to bunt is due to the presence of a physiologic form of the pathogene previously not prevalent in durum-growing regions.[6] Field observations during the season of 1930 also confirm the conclusion that durum wheats, as a class, are no longer resistant to bunt, as evidenced by the fact that the amount of bunt in commercial fields of Mindum ranged from a trace to 51 per cent (Table 2).

The Litchfield, Minnesota, collection of *Tilletia tritici* produced 23.6 per cent of bunt in 1929 and 16.1 per cent in 1930 on Vernal, while all

[5] Data on commercial wheat fields were obtained by the writer during a survey conducted by the Agricultural Extension Division of the University of Minnesota in cooperation with the Plant Disease Survey, United States Department of Agriculture.

[6] Holton, *l. c.*

TABLE 2.—*Smut produced by Tilletia levis and T. tritici in 31 commercial fields of Marquis and Mindum wheat and Vernal emmer in three widely separated counties in Minnesota 1930*

Variety of grain	Species of Tilletia	Percentage of smut
Marquis	levis	40
"	"	40
"	"	35
"	"	38
"	"	35
"	"	5
"	"	2
"	"	4
"	"	5
"	"	3
"	"	4
"	"	4
"	"	3
"	"	4
"	"	4
"	"	14
"	"	2
"	"	5
Mindum	tritici	40
"	"	51
"	"	5
"	"	8
"	"	5
"	"	4
"	"	7
"	"	5
"	"	11
"	"	tr.
"	"	3
"	"	tr.
Vernal	"	40

other varieties inoculated, except Kota, were highly resistant. In the one field of Vernal observed in 1930, 40 per cent of the heads were smutted with *T. tritici* (Table 2). It is evident that additional experimental data and field observations support the contention that Vernal is no longer resistant to bunt.

Marquis, a common wheat previously markedly resistant to bunt, is now known to be quite susceptible. In 1929 a field of Marquis near Ivanhoe, Minnesota, was reported heavily infected with bunt, approximately 40 to 50 per cent. A sample of bunt was collected from this field, identified as

Tilletia levis and used in the inoculation experiment in 1930. This collection produced 23.3 per cent of bunt on Marquis and proved extremely virulent on Kota and Ceres and moderately virulent on Marquillo (Table 1). When commercial fields of Marquis were examined in 1930 it was not uncommon to find very much higher percentages of bunt than had been reported prior to 1929. In 5 of the 18 fields examined there was 35 per cent or more of bunt (Table 2). It is obvious that Marquis can no longer be considered highly resistant to all forms of bunt, and it is probable that the increased amount of bunt in it is due to a hitherto unknown physiologic form of *T. levis* that, until recently, has not been prevalent in regions where Marquis wheat is grown.

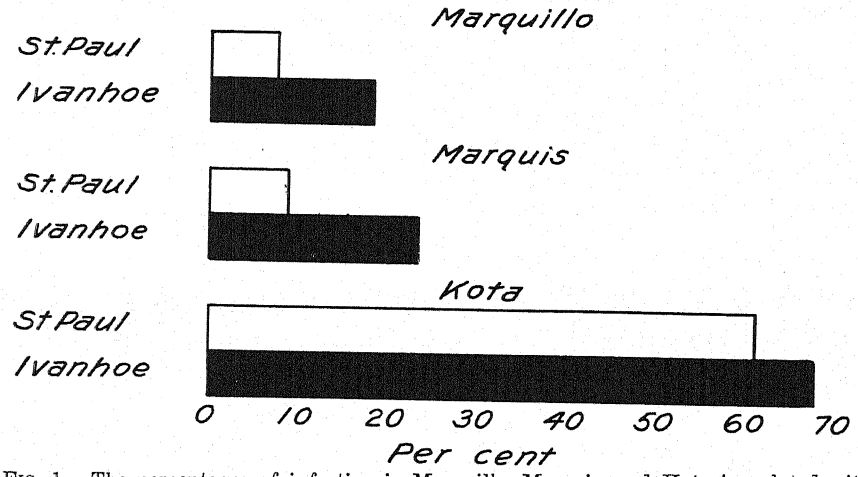


FIG. 1. The percentages of infection in Marquillo, Marquis, and Kota inoculated with spores of two collections of *Tilletia levis* from Minnesota.

It is obvious from the results summarized in table 1 that there are two distinct physiologic forms[7] of *Tilletia levis,* based on their pathogenicity for the varieties inoculated. The form from St. Paul, Minnesota, is extremely virulent on Kota and Ceres, while Marquis and the other varieties tested were either highly resistant or virtually immune. This is in accordance with the results obtained by Rodenhiser and Stakman.[8] The form from Ivanhoe, Minnesota, also is extremely virulent on Kota and Ceres and, in addition, attacks Marquis and Marquillo heavily (see Table 1 and Figure 1). Hence, it is considered distinct from the St. Paul form because of its greater virulence on Marquis and Marquillo.

[7] The propriety of designating these collections as physiologic forms is discussed on page 354 of the writer's first paper (*l. c.*).

[8] Rodenhiser, H. A., and E. C. Stakman. Physiologic specialization in *Tilletia levis* and *Tilletia tritici.* Phytopath. 17: 247–253. 1927.

Field observations, which are summarized in table 2, indicate that all the fields of Marquis examined were infected with *Tilletia levis,* while all the fields of Mindum, as well as the single field of Vernal, were infected with *T. tritici.*

This observation is borne out by experimental evidence inasmuch as the results in table 1 show clearly that the highest percentages of smut on Marquis and Marquillo are produced by *Tilletia levis,* while the highest percentages of smut on Mindum and Vernal are produced by *T. tritici.* Similar evidence has been presented by Brentzel and Smith.[9]

It is perfectly evident that new physiologic forms of *Tilletia levis* and *T. tritici* have been and apparently still are making their appearance in the hard-red-spring-wheat region. These forms are quite distinct from each other in pathogenic capabilities. Whether these forms were introduced into the region from other regions, whether they arose as a result of mutations, or whether they arose as a result of hybridization between existing forms is not known. It has been shown by Stakman, Christensen, Eide, and Peturson[10] that *Ustilago zeae* (Beckm.) Ung. mutates abundantly and that some of the mutants differ sharply from their parents in pathogenicity. It was shown also by these authors that hybridization is extremely common within the species. There is no reason whatever why new forms of *T. levis* and *T. tritici* should not originate through these perfectly natural processes. It is possible that they may originate as a result of hybridization between different physiologic forms or possibly even between the two species. It also is entirely possible, of course, that they may have been introduced from other regions. Whatever the mode of origin, it is perfectly apparent that new physiologic forms of the two bunt species do appear and that varieties which have been resistant in the field hitherto may be very susceptible to new forms. The practical importance of this fact is perfectly obvious and is very clearly apparent in connection with the bunt situation. For many years, the durums remained almost free from bunt in the upper Mississippi Valley and Great Plains region. It was shown, as a result of extensive inoculations, that virtually all of the durum varieties actually were highly resistant to those forms of the bunt species which seem to be most prevalent, but the durums can no longer be considered resistant for practical purposes, as they are very susceptible to certain physiologic forms that are widely enough distributed to cause real epiphytotics of bunt in durum wheats. Furthermore, Marquis, extensively grown in the spring-wheat region since about 1915, has also been highly resistant to bunt in the field. It was also

[9] Brentzel, W. E., and Ralph W. Smith. Varietal resistance of spring wheats to bunt. N. Dak. Agr. Exp. Sta. Bul. 231. 1929.

[10] Stakman, E. C., J. J. Christensen, C. J. Eide, and Bjorn Peturson. Mutation and hybridization in *Ustilago zeae.* Minn. Agr. Exp. Sta. Tech. Bul. 65. 1929.

decidedly resistant to all collections of bunt with which it was inoculated artificially in the experimental plots at University Farm, St. Paul, Minnesota, for at least 10 years. During the summer of 1930, however, the writer showed that this variety is very susceptible to at least one physiologic form of *T. levis,* which also has become widely enough distributed geographically to cause considerable losses to Marquis in the field. Marquillo, which because of its parentage would be expected to be highly resistant and which actually has been resistant when grown in the field, also is susceptible to at least one physiologic form of *T. levis.* From previous experience it seems likely that this variety also may become rather heavily bunted after it has been grown commercially for a sufficiently long time to permit the accumulation of inoculum on the seed. It seems very clear, therefore, that the bunt situation is continually changing and that varieties, resistant in one locality, may be susceptible in another. Furthermore, varieties may be resistant for a number of years in the same locality only to succumb to attacks of bunt when new physiologic forms make their appearance. The exact nature of resistance of varieties of wheat to bunt is not clearly understood. It seems probable, however, that varieties in order to be long useful from the standpoint of bunt resistance may have to have some sort of structural or functional resistance that will enable them either to resist or escape the attacks of the pathogene. It seems highly desirable, therefore, to make basic studies on the nature of resistance. In the meantime the writer considers it of the utmost importance to continue investigations toward the perfecting and simplification of seed treatments. It would seem extremely important to continue very vigorously the campaign for the treatment of all seed wheat and particularly of new and improved varieties. One can never be sure of the bunt situation at any given time, and it would seem to be the part of wisdom, therefore, to use to the fullest possible extent the method of bunt control which is known to be efficacious, namely, seed treatment, and, in some regions, crop rotation.

SUMMARY

1. The virulence of four collections of *Tilletia tritici* and two collections of *T. levis* was compared on ten varieties of Triticum.

2. The results with *Tilletia tritici* confirmed those already published[11] Obviously, the recent epiphytotics of bunt in durum wheats are caused by a physiologic form which has not been prevalent until fairly recent years. Experimental evidence and field observations in 1930 also confirm the conclusion drawn from results obtained in 1929 that Vernal, previously resistant, now is susceptible to bunt, because of the appearance of a form of *T. tritici* previously unknown.

[11] Holton, *l. c.*

3. Two physiologic forms of *Tilletia levis* were identified on the basis of their reaction on Kota, Ceres, Marquis, and Marquillo. The St. Paul, Minnesota, collection represents one form which is distinguishable by its high degree of virulence on Kota and Ceres and low virulence on Marquis and Marquillo. The collection from Ivanhoe, Minnesota, represents another form which attacks Marquis and Marquillo, as well as Kota and Ceres, with a high degree of virulence.

4. Field observations have confirmed experimental evidence that recent outbreaks of bunt in Marquis wheat are due to the presence of a hitherto undescribed physiologic form of *Tilletia levis*.

5. Under field conditions *Tilletia levis* is the predominant species occurring on Marquis, and *T. tritici* is the predominant species on Mindum and Vernal. This observation is confirmed by experimental results which show that Marquis is quite susceptible to *T. levis* and highly resistant to *T. tritici* while the reverse is true with Mindum and Vernal.

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THE EFFECT OF ULTRA-VIOLET LIGHT RADIATIONS ON THE VEGETATIVE GROWTH OF WHEAT SEEDLINGS AND THEIR INFECTION BY ERYSIPHE GRAMINIS[1]

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EXPERIMENTAL PROCEDURE

Source of light: The lamp used was a quartz mercury-vapor lamp (Hanovia Artificial Alpine Sun, 2.5 amps., 210 volts direct current type). An interval of 3 minutes was allowed to elapse after lighting up before using the lamp, in order to allow it to become steady. At the intensities employed there was very little heat effect, as registered with an ordinary mercury thermometer.

The objects to be irradiated were grouped directly under the light, the outer pots or boxes being tilted slightly to allow the light to fall normally on the leaves. Distances were measured from the center of the quartz tube to the tips of the plants.

Source of infection: The plants were kept in a greenhouse where 100 per cent infection had occurred in susceptible varieties for several years past and where non-treated wheat plants were already showing such infection.

Material used: The varieties of wheat used were Squarehead's Master, Little Joss, American Club (susceptible), and Persian Black (immune).

PRELIMINARY EXPERIMENTS

A few preliminary experiments were conducted in order to determine the quantity of light that would be effective in reducing the infection of the plants by the mildew without greatly damaging the plants themselves; and also to determine whether irradiation with ultra-violet light would render the immune variety, Persian Black, susceptible to infection. Of these experiments the most conclusive was as follows:

Seeds of Persian Black, Little Joss, and Squarehead's Master were sown in earth and 24 days later were placed in 4½-inch pots, 4 plants per pot. After an interval of 2 days, to allow the plants to become established, 2 pots of each variety were irradiated daily for 3, 10, and 15 minutes, respectively, at 24 inches from the light, and 2 pots of each variety were left

[1] This work was carried out at the suggestion of Dr. W. A. R. Dillon Weston, as a continuation of his own work on the action of ultra-violet light on some specific fungi.[2] To him the writers wish to express their gratitude for the suggestion and for the loan of apparatus and material; also to E. T. Halnan, Esq., for his advice and assistance.

[2] Weston, W. A. R. Dillon. The fungicidal action of ultra-violet radiation. Phytopath. 20: 959–965. 1930.

non-treated as controls. The treatment was continued 14 days, and the plants were examined within 24 hours after the last irradiation and again a week later. Before irradiation commenced, with the exception of the Persian Black seedlings, all the plants were badly infected with mildew.

First examination: Persian Black. All plants perfectly free from mildew. Those treated 3 minutes no scorching effect, 10 and 15 minutes badly scorched.

Little Joss and Squarehead's Master. Controls, all badly mildewed, every leaf attacked. Treated 3 minutes, mildew slight and only on old, lower, yellowed leaves on surface away from light; new leaves free. Treated 10 and 15 minutes, almost no mildew, leaves badly scorched. In every case Squarehead's Master was more highly infected than Little Joss.

Second examination: Persian Black still noninfected. Squarehead's Master and Little Joss plants all badly infected.

This experiment shows that by a suitable irradiation it is possible to render infected plants almost free from disease, but, at the concentrations so far employed, the plants themselves were severely injured. It was therefore determined to discover if, commencing with plants free from mildew, suitable irradiation would keep them clean without deleterious effect on their growth.

SUBSEQUENT EXPERIMENTS: EXPERIMENT NO. 1

Little Joss and Persian Black: Pots of Little Joss and Persian Black seedlings, about 11 cm. high and free from mildew, were treated 5 days as follows:

1, 5, 10	minutes	once	a	day	at	24	inches	from	the light.
1 and 5	"	twice	"	"	"	24	"	"	"	"
1	minute	once	"	"	"	48	"	"	"	"
1	"	twice	"	"	"	48	"	"	"	"

There were 2 pots of each of these treatments of both varieties, and, in addition, 3 pots of each variety were left as controls.

The plants were examined the day after the last irradiation and again a fortnight later.

First examination: Persian Black. All plants free from mildew. No scorched plants except in those cultures treated 5 and 10 minutes.

Little Joss. Controls, all plants badly infected with mildew; no scorch.

Treated.

1	minute	at 48	inches	once.	Mildew just showing. No scorching.
1	"	" 48	"	twice.	No mildew. No scorching.
1	"	" 24	"	once.	Mildew very slight. No scorching.
1	"	" 24	"	twice.	No mildew. No scorching.

5	"	"	24	"	once.	"	"	Slight scorching.
5	"	"	24	"	twice.	"	"	Badly scorched.
10	"	"	24	"	once.	"	"	Slight scorching.

Second examination: Persian Black. All plants still free from mildew. It was very noticeable that although all the plants were exactly alike at the commencement of the experiment, those irradiated 1 minute a day at 48 inches were all much stronger and taller than any of the others.

Little Joss. All plants badly mildewed; the controls, nearly killed. No noticeable difference in height between any of these plants, as in the case of Persian Black.

SUMMARY

The general result of this experiment seems to show that it is possible to render the plants free from mildew without causing any visible harmful effects on the plants themselves. When irradiation was discontinued the plants became infected. The effect of irradiation twice a day seems greater than irradiation for the same total time once a day.

In order to test the results of Experiment No. 1 more accurately, the following experiment was devised.

EXPERIMENT NO. 2

Three boxes of wheat seedlings, approximately 8 cm. high and about 300 plants per box, one box, each, of American Club, Little Joss, and Persian Black, were each divided into three approximately equal portions. One of these divisions was left untreated as a control, and the other two were irradiated, the one for 1 minute and the other for 3 minutes a day for 15 days, at 48 inches from the lamp. At the commencement of the experiment the seedlings were all free from mildew.

Twelve hours after the last irradiation most of the seedlings were taken up and an average sample of 100 from each division in each box was carefully measured for height and examined for mildew. Those plants upon which not a single pustule of the fungus could be seen by the naked eye were recorded as clean.

TABLE 1.—*Average height, in centimeters, of the seedlings of American Club, Little Joss, and Persian Black treated 1 minute and 3 minutes with ultra-violet light*

Variety	Control	Treated 1 minute	Treated 3 minutes
American Club	23.5	24.8	22.1
Little Joss	24.1	27.5	23.5
Persian Black	21.8	21.9	21.4

The Persian Black seedlings became "lodged" so that the light was not equally distributed on all the plants in each section, and therefore the re-

sults cannot be quite comparable with those obtained on the other two varieties.

TABLE 2.—*Percentage of American Club, Little Joss, and Persian Black wheat plants infected by mildew following exposure to ultra-violet light for periods of 1 and 3 minutes, respectively*

Variety	Number clean	Number infected	Percentage infected
American Club			
Control	22	78	78
1 minute	26	74	74
3 minutes	90	10	10
Little Joss			
Control	0	100	100
1 minute	9	91	91
3 minutes	48	52	52
Persian Black			
All clean			

Those seedlings left in the boxes were kept without further treatment for a week and then examined, when it was seen that the Persian Black plants were still not infected, while those of the other two varieties were all badly attacked by mildew.

SUMMARY

With American Club and Little Joss 1 minute of irradiation gave increased growth but only slightly reduced the percentage of infection; while irradiation for 3 minutes reduced the growth but greatly reduced the percentage of plants infected by mildew; but when irradiation ceased the plants again became infected.

In the case of Persian Black the effect on growth was not so marked, and its immunity from mildew was not affected.

DISCUSSION

In these experiments it was found possible to give the plants an amount of ultra-violet light irradiation which gave fairly effective control of the fungus without causing much damage to the host.

The light apparently acts directly on the fungus mycelium, killing it or rendering it dormant. The action is not an indirect one affecting the metabolism of the host in such a way as to render it immune or resistant. This is shown by the fact that the fungus was suppressed only on the side of the leaves exposed to the light. (In some of the experiments the pots were turned about so as to expose all surfaces to the light, and in these cases the fungus was killed on both sides of the leaves.) Also, when irradiation was

stopped the plants became as readily and severely infected as before. Since, however, certain quantities of light were found to give increased growth, this would have an indirect effect in enabling the plants to flourish in spite of slight infection.

Smaller quantities of light were required to keep clean plants free from disease than to kill the fungus on infected plants. This probably means that the young hyphae are more susceptible to ultra-violet irradiation than are the older mycelia.

It was found impossible, even when using quantities of light which produced severe scorching, to break down the resistance to infection of the variety Persian Black. This is contrary to what might have been expected, since cutting or burning leaves of cereals will often break down their resistance to attacks of mildew.[3]

In view of the fact that certain quantities of irradiation gave increased growth of the wheat plants (which confirms a result obtained by Delf, Ritson, and Westbrook),[4] it would have been interesting to correlate the quantity of ultra-violet-light irradiation to which the plants were subjected with the quantity occurring in normal summer sunshine. An attempt was made to do this, but it was found impossible. It is a well-known fact, however, that in England the hotter and drier the summer, the greater the yield of wheat. This is borne out by the following figures: In 1929 the wheat yield averaged 19.1 cwt. [31.8 bushels] per acre and the weather conditions were exceptionally hot and dry and the incidence of mildew was slight.[5] This year the weather has been wet and the incidence of mildew fairly great; the yield is estimated at only 15.8 cwt. [26.3 bushels] per acre. The 10 years' average is 17.7 cwt. [29.5 bushels]. It is probable that in an English summer the amount of ultra-violet irradiation is never sufficiently high to give the maximum yield of which the wheat crop is capable. But, the amount may be sufficient, as in 1929, to suppress the growth of the fungus, and this is possibly one of the factors influencing the yield; whereas, in 1930, the amount was so low that the fungus flourished and the crop thereby suffered.

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[3] Salmon, E. S. On Erysiphe graminis DC. and its adaptive parasitism within the genus Bromus. Ann. Mycol. 2: 307–343. 1904.

[4] Delf, E. M. The effect on plants of radiations from a quartz mercury vapour lamp. Brit. Jour. Exp. Biol. 5: 138–154. 1927.

[5] Ministry of Agriculture and Fisheries (England). Monthly Agr. Rpts. 1929–1930.



TURGESCENCE AND RUPTURE OF POTATO TUBER

H. G. MacMillan

A condition arose recently in a field of potatoes under irrigation which gave opportunity for a limited study of some effects of high turgescence in tubers. The potatoes were of the Bliss Triumph variety. They were of excellent quality and the yield was satisfactory, as 320 bushels per acre of marketable tubers were harvested. However, nearly half of the tubers showed an unusual condition of injury.

The injury consisted of newly made cracks, fairly deep into the tuber flesh and of unequal and indeterminate extent, often shaping into curious and fantastic fissures. There was no regular design in the rupture. The usual depth was $\frac{1}{4}$ to $\frac{3}{8}$ of an inch below the surface. Some tubers showed a single crack an inch or more in length and quite straight; some were curved on the surface immediately presented to view; while others followed around the tuber, virtually ringing it, as though the tuber were about to break in two pieces. The majority were unequally radial, the cracks extending out from a central point in distorted star shapes. There were still other designs. When freshly made, the openings were white and gleaming within but soon became gray, as the surface dried and shrank. The injury was being caused in digging. The tubers in the ground were clean, bright, and free from cracks. The process of digging bumped them over the elevator of the digger, against each other, and finally out behind with a fall of a foot or more to the ground. The fall from the digger where one tuber struck another already on the ground was enough to cause the bursting and rupture of one or both of them. The soil was a moist sandy loam, free from stones and clods, or the damage would have been greater. The process of picking, sorting, and sacking, all of which went on in the field soon after digging, added further damage.

The condition of high turgidity and the resisting tension which the tuber offered could not be measured but could be illustrated. Upon pricking the surface with a sharply pointed knife to the depth of $\frac{1}{4}$ inch the fissures would form instantly, some for a length of 2 inches or more. Tubers were found which, on being once quickly stabbed with a pin, would break out a star-shape rupture with radii as much as an inch long. Yet no tubers could be found that, on being carefully taken from the ground, showed any trace of rupture or indication of this tendency.

A number of tubers were dug, taken to the laboratory in a closed vessel, brushed carefully to remove soil particles, and weighed. They were laid on a table freely exposed to the air. At 2-day intervals they were

reweighed and two or more subjected to pricking with a knife or dropped a distance of 12 inches to the table. There was a gradual decline in weight and a corresponding diminution in the extent of the rupture. It was necessary to discard ruptured tubers. At the end of 8 days no rupturing occurred except on application of unusual and unwarranted violence. The few remaining tubers, which appeared as sound and fresh as when dug, were weighed. The weights are shown in table 1.

TABLE 1.—*The weights of 8 tubers as dug in the field, the weights after air-drying for 8 days, the difference in grams, the percentage loss in weight for each tuber, and averages*

Tuber no.	Weight as dug	Weight after 8 days air drying	Weight loss	Weight loss
	Grams	*Grams*	*Grams*	*Per cent*
1	521.65	484.70	36.95	7.083
2	234.81	213.41	21.40	9.113
3	231.95	216.42	15.53	6.695
4	216.30	208.37	7.93	3.666
5	186.32	181.21	5.11	2.742
6	170.70	158.90	11.80	6.912
7	119.22	114.02	5.20	4.361
8	63.29	60.00	3.29	5.198
Average	218.03	204.63	13.40	6.145

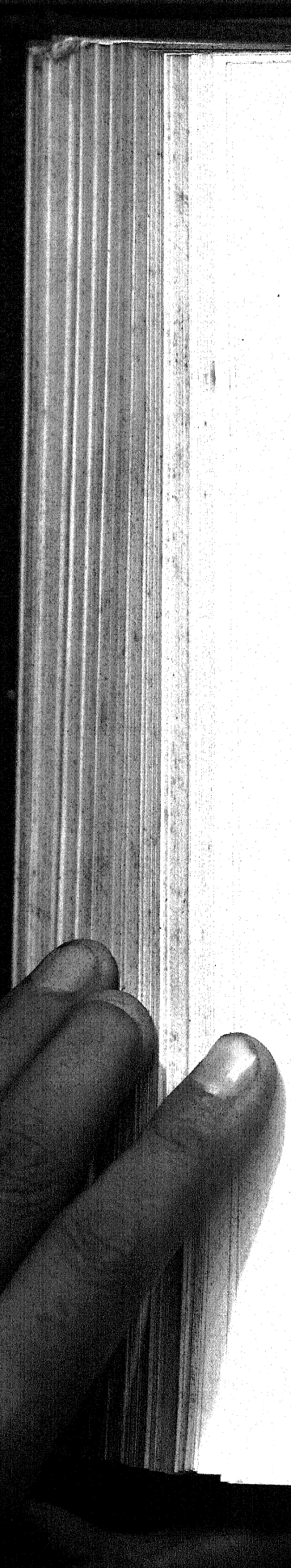
The day after digging began and 2 days prior to the call to the field, a 0.30 inch rain fell. Following the rain a moderate frost killed the tops of the vines. The large amount of water available to and taken up by the uninjured roots evidently was forced into the tubers. That they were unequally filled with water of turgescence was indicated by the varying damage in the field and by the unequal shrinkage of the tubers weighed and dried to the nonbursting point. The heavier tuber, weighing a pound or more, would be liable to greater damage than the smaller one falling the same distance. The area of impact would be about the same in each case, but the falling weight behind it put a greater strain on the contact area of the larger potato. The larger tubers showed more damage than the smaller. The table shows that of the few tubers reaching a final weight it was necessary for them to lose an average of 6.14 per cent of their weight as dug to be restored to an approximately normal condition. If the dried weight is regarded as the normal weight it would appear that an increase of approximately 6.5 per cent in weight due to excess water is possible without damage to the potato, except as the result of rough handling. Careful selection of individual tubers would materially raise this amount,

as would be indicated from the weight of tuber No. 2. With the decline and death of the vines no doubt the tubers would have soon lost water to the point where they could have been dug with safety. Postponed digging would have controlled the trouble.

The freshly exposed ruptured tissue began at once to dry and to form a covering of dried and collapsed cells. The open area exposed to air was $\frac{1}{4}$ to $\frac{3}{8}$ of an inch deep. Callous tissue, however, formed more deeply, extending $\frac{1}{2}$ inch or more below the surface. This indicated that the deeper-lying tissues were wrenched and torn, though they were not visibly spread apart. The tissues at the surface of the tuber, probably inclusive between the epidermis and the vascular layer, seemed to restrain the rupture. The pressure appeared to exist only in the pith or below the vascular region. Cutting the tubers revealed no water-soaked areas within. All tissues were normal in appearance.

No additional cases of injury equal to the one described have been observed. Small ruptures have been observed many times in irrigated potatoes, where the cracks have been $\frac{1}{2}$ inch long or less and shallow. The usual result of these ruptures is the development of small pads of corky or callous tissue within the flesh. The former are soon lost to view. In the case described there was no opportunity for extended study. The potatoes were soon dug and moved. To attempt to duplicate the conditions experimentally would have been difficult, if not impossible, and, in view of the restricted local damage, entirely unwarranted.

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RESISTENCIA COMPARATIVA A LA TILLETIA LEVIS KÜHN, DEL TRIGO, EN LA ARGENTINA[1]

ING°. AGR°. RAIMUNDO NIEVES[2]

OBJETO

Se iniciaron estos ensayos en 1928, como tarea preliminar de un vasto programa de investigación, tendiente a la creación de variedades de trigo muy resistentes a la caries (bunt), y de alto valor agricola-industrial.

Es sabido que existen métodos de desinfección absolutamente eficaces para prevenir esta enfermedad y desde la aparición de los métodos de curación "en seco" y de los polvos anticriptogámicos, puede decirse que la profiláxis de la caries es fácil, rápida y barata.

No obstante, la desidia de muchos agricultores y muchas veces la mala aplicación de los métodos curativos, hacen que esta enfermedad que pudiera extirparse del pais—asi como el carbón volador (loose smut)—por la sola y rigurosa aplicación de leyes profilácticas adecuadas, aparezca constantemente, con mayor o menor intensidad segun regiones.

Estas circunstancias, son las que quizás han movido a afirmar, a investigadores tales como Gaines, Tisdale et al. y Tingey, que pese al continuo mejoramiento de los métodos de curación, no se ha logrado un control satisfactorio de la plaga en regiones trigueras muy afectadas y que se hace entonces necesario concentrar esfuerzos, en la creación de variedades inmunes, ya que con su utilización, se puede alcanzar tranquila y seguramente, lo que quizás no lograra el empleo de leyes conminatorias.

DAÑOS

Los perjuicios que ocasioná la caries son de distinto orden:

(1) Disminuye el rendimiento unitario del cereal;

(2) Desvaloriza el producto;

(3) Puede provocar incendios en las trilladoras. Diversos autores afirman, que la mayoria de las explosiones en las trilladoras se producen en la trilla de trigos muy carbonudos;

[1] Contribución de la Estación Experimental de Guatraché (Pampa Central), de la Dirección General de Enseñanza y Fomento Agricola, del Ministerio de Agricultura de la Nación. Republica Argentina.

[2] Director de la Estación Experimental de Guatraché.

(4) Obliga a cambiar la semilla o al empleo forzoso de anticripto gámicos.

A su vez, dichos perjuicios, varian con el pais o regiones que se consideren, porque su distribución geográfica y virulencia, se hayan muy influenciados por el clima, naturaleza del suelo y caracteristicas biológicas de los trigos cultivados.

Dickson (11), establece que las pérdidas son apreciables en Kansas, mientras que en Wisconsin, la caries seria desconocida. Boerger (3), sin precisar el monto de las pérdidas sufridas por el Uruguay, establece que mientras la infección puede ser considerable en las siembras tempranas, llega a ser nula en las siembras muy tardias que coinciden además con periodos lluviosos.

Gaines (17), estima en 15 por ciento la pérdida anual sobre los trigos de invierno en solo Los estados de Washington, Oregon e Idaho, representando la misma un valor de 10 millones de dólares.

Tingey (47), estima que las pérdidas son muy grandes en los estados de las Rocky Mountains y cita que en 1925, treinta por ciento del trigo de Utah e Idaho, era "carbonudo."

Stevens y Hall (44), establecen que la pérdida anual en los EE.UU. por caries del trigo, es alrededor de 25 millones de bushels, a la que debe agregarse las pérdidas por explosiones de trilladoras, estimadas en un millón de dólares en dos años.

Marchal (30), la considera la enfermedad mas grave del trigo en Belgica y estima las pérdidas en 5 por ciento del valor total de la cosecha.

Delacroix y Maublanc (10), tambien la consideran una enfermedad muy extendida en Francia.

En Alemania, constituye una enfermedad de importancia económica, y a juzgar por los ensayos de Roemer, que transcribe Gaines (19), existen en ese pais formas fisiológicas de *Triticum tritici* Bjerk. (Wint.) de extrema virulencia, a las que han sucumbido trigos considerados inmunes en Estados Unidos.

En Palestina segun Reichert (40) habrian formas fisiológicas especialmente virulentas sobre los *Triticum durum* Desf. que en cambio no afectarian mayormente a los *T. vulgare.*

Algo semejante ha constatado recientemente Holton (23) en Minnesota, lo que le permitió afirmar, que las recientes epidemias de Caries sufridas por los *T. durum,* considerados generalmente muy resistentes a las Tilletias, en la "hard red spring wheat area," se deberian a la aparición de una nueva forma fisiológica, introducida a la region, o producida "in situ" por mutación o hibridación.

En la Argentina no se ha realizado una investigación completa tendiente a precisar las pérdidas anuales por las Tilletias, ni se ha establecido el area

de dispersión de las dos especies. Tampoco se han clasificado regiones de mayor o menor virulencia, ni establecido la existencia de formas fisiológicas especializadas, realizándose solo esfuerzos aislados en el exámen de aspectos particulares del problema.

No obstante puede afirmarse que en las regiones frias y con lluvias medianas a escasas, como son La Pampa, San Luis y el Oeste y Sud de Buenos Aires, las pérdidas son de consideración.

En las regiones trigueras lluviosas: Santa Fé, Entre Rios, Córdoba, Norte y Este de Buenos Aires, las Tilletias parecen no encontrar condiciones muy propicias para su evolución, siendo en cambio más común y perjudicial el *Ustilago tritici* (carbón volador)

ANTECEDENTES EN LA LITERATURA AGRICOLA

Investigaciones similares han sido realizadas por Farrer (15 y 16), Darnell-Smith (8) y McAlpine (31), en Australia; per Tubeüf (49), Kirchner (29) y Hecke (22), en Alemania; por Vavilov (51) en Rusia; por Donkin (12) en Sud Africa; por Gaines (17), Tisdale et al. (48), Stakman, Lambert y Flor (43), Tingey (47), Brentzel y Smith (4), etc. en EE. UU.

En nuestro pais, en 1928, Williamson (53) comunicó los resultados obtenidos en infecciones experimentales en un limitado número de variedades. Brunini (5) en 1930, comunica los resultados de los ensayos por él realizados en 1928 y 1929, con 12 trigos, todos ellos del grupo *T. vulgare,* y cultivados extensamente en el sud de Buenos Aires. Con excepción del Kanred, las restantes 11 variedades son de hábito primaveral o intermedio y se manifiestan muy susceptibles con promedios que oscilan entre 24 a 75 por ciento.

El Kanred se comporta como resistente, dando solo 12.2 por ciento de infección, como promedio de 5 repeticiones.

MATERIAL

Los trigos ensayados comprendian un total de 154 variedades pertenecientes a 5 subespecies. De ellas, 84 son de hábito invernal y 70 de hábito primaveral o intermedio. Los cuadros 1 y 2, presentan el detalle.

METODOS

La semilla se infectó poco antes de la siembra, con esporos de *Tilletia levis,* cosechados el año anterior sobre la variedad Tuzela.

Se empleó un exceso de esporos, agitándolos con la semilla, dentro de un tamborcito durante 5 minutos. Luego el polvo negruzco sobrante se eliminaba por tamizaje y las semillas ennegrecidas se alojaron en sobres numerados.

Las siembras se efectuaron en parcelas corridas, a 15 x 15 cm. entre plantas y en 3 épocas. Con excepción de 58 variedades, en las restantes 96, se obtuvieron de 160 a 180 plantas para cada una. En el cuadro 3, se presentan tabuladas, la distribución de las parcelas en cada época, el número de siembras individuales y el total de espigas examinadas.

Las variedades Florence y Tuzela, elejidas como testigos, la primera por su resistencia y la segunda por su enorme susceptibilidad, se repitieron en todas Las épocas y dentro de cada época en condiciones distintas.

CUADRO 1.—*Con la distribución de las variedades por su origen y hábito*

	Siembras en				
	Abril–Mayo		Julio		
Variedades	Hábito invernal	Hábito primaveral	Hábito invernal	Hábito primaveral	Totales
Comunes	10	4	...	4	18
De "pedigree"	15	2	...	6	23
Nuevos hibridos	13	...	...	51	64
Nuevas *L. puras*	42	...	4	3	49
Totales	80	6	4	64	154

CUADRO 2.—*Con la distribución de las variedades ensayadas, según su naturaleza genética*[a]

TRIGOS A 21 PARES DE CROMOSOMAS	
	v. graecum Körn. (tipo: Sary Maghis)
	v. erythrospermum Körn. (tipo: Kanred)
T. vulgare Vill.	*v. ferrugineum* Körn. (typo: Record)
143 vars.	*v. albidum* Körn. (tipo: Florence)
	v. lutescens Körn. (tipo: Ulka)
	v. milturum Körn. (tipo: Ruso pelón)
T. compactum Host.	
2 vars.	*v. icterinum* Körn. (tipo: Ballod Cheg.)
	v. melanopus Körn. (tipo: Tchersnouska)
T. durum Desf.	*v. coerulescens* Körn. (tipo: Tchersnoostaia)
6 vars.	*v. hordeiforme* Körn. (tipo: 45 b)
	v. erythromelan Körn. (tipo: Chargarod)
T. Turgidum L.	
1 var.	*v. pseudocervinum* Körn. (tipo: Alaska)
T. persicum Vav.	
1 var.	*v. fuliginosum* Zhk. (tipo: Fuliginoso)
T. dicoccum Schubl.	
1 var.	*v. atratum* Körn. (tipo: Black Winter)

[a] La clasificación adoptada es la consignada por el professor N. I. Vavilov, Jefe del Instituto de Botánica Aplicada y Mejoramiento de las Plantas, de Rusia, en sus "Studies on the Origin of Cultivated Plants" Leningrado, 1926.

CUADRO 3.—*Presentando la distribución de las parcelas en cada época de siembra, el número de siembras individuales, y el total de espigas examinadas*

Fecha de siembra	Numero de parcelas	Numero de variedades	Numero de siembras	Plantas por variedad	Numero de espigas
Abril 30	62	28	4,960	160	13,446
"	21	14	840	40	2,517
Mayo 5	47	44	1,880	40	13,455
Julio 17	68	68	12,240	180	29,776
Totales	198	154	19,920	.....	59,194

Se consideró cuidadosamente, el regimen climatérico durante la germinación (estas observaciones las hemos comunicado en otro trabajo, 34).

Al verificarse el recuento de las espigas enfermas se observó el criterio, que una sola espigueta infectada bastaba para considerar en ese estado a toda la espiga. Este criterio riguroso solo abulta los porcentajes en las variedades muy resistentes, que son las que generalmente presentan espigas en esas condiciones.

Los porcentajes dados en las planillas que se acompañan expresan la proporción de espigas enfermas sobre el total. No se consideró el porcentaje en "plantas infectadas," ni el porcentaje de "infección individual," tal como los presentan Gaines (17) y nosotros en otros trabajos (34 y 36).

Advertencia.—Los resultados aqui comunicados se obtuvieron en 1928.

En 1929, repetimos éstos ensayos, pero la terrible sequia que aflijió La Pampa, ese año, terminó con ellos.

En 1930, se han vuelto a repetir, multiplicando el número de repeticiones y el de variedades bajo control.

El deseo de no demorar mas la publicidad de resultados que conceptuamos interesantes, por tratarse del primer ensayo realizado en gran escala en la Argentina, justifica su presentación en este momento.

RESULTADOS

(I) *Influencia sobre la infección, de la temperatura y humedad durante la germinación. Sus relaciones con las épocas de siembra.*—Como la temperatura y humedad durante la germinación, se hayan estrechamente vinculadas, al régimen climatérico del més en que se efectúa la siembra, "prima facie," podemos referir la variabilidad de la infección, como variable dependiente de la época de siembra.

En ese sentido, en otro trabajo (34) y aprovechando las cifras de estos ensayos, llegamos a la conclusion:

CUADRO 4.—*Con la lista clasificada de las variedades y porcentajes de infección, en el lote No. 1, de las siembras de Abril 30 de 1928*

Variedad	Naturaleza	Clasificacion	Total espigas	Espigas "cariadas"	Porcentajes
Florence	L. Pura H.	B.SMD.P.	228	18	7.8
"	"	"	345	25	7.2
"	"	"	223	11	4.9
Lin Calel	L. Pura	C.D.I.	316	298	94.3
Marquis	L. Pura H.	"[a]	354	217	61.3
Ruso Pelon	Sel. Masal	"	450	430	95.5
"	"	"	433	389	89.8
Hibrido de Kalt	L. Pura H.	C.SMD.P.	183	93	50.8
"	"	"	170	76	44.8
Kansas	Sel. Masal	C.D.I.	446	105	23.5
"	"	"	380	51	13.4
Ruso Aristado	"	"	413	403	97.5
"	"	"	337	326	96.8
Era	L. Pura H.	C.SMD.I.P.	242	125	51.6
"	"	"	248	117	47.1
Principe de Gales	"	"	242	134	55.3
"	"	"	173	101	58.4
Maravilla	"	"	284	271	95.4
"	"	"	255	233	91.3
Williamson	L. Pura	C.D.I.	481	381	79.2
"	"	"	377	262	69.4
Pelon Colorado	Sel. Masal	C.D.I.P.	417	354	84.8

[a] El Marquis es considerado en el Canada y EE.UU., un trigo de Primavera. Como en nuestra región, se comporta como trigo de habito invernal, lo hemos clasificado en esa categoria.

que el maximún de condiciones favorables a la infección, parece registrarse en las siembras otoñales de los trigos de invierno—en Abril y Mayo— siendo interesante constatar que Tingey (l. c.), llega a las mismas conclusiones en sus investigaciones en Utah.

Analizando minuciosamente el problema, se vé que los factores que mas influencian la infeción embrionaria son: la temperatura y la humedad. Estudiando estos puntos en la comunicación antes citada, llegamos a las siguientes conclusiones:

1) que las infecciones menores se registran cuando el trigo germina a temperaturas muy bajas (6 a 7 grados C.) regimen termométrico que en la región se tiene en los meses de Junio y Julio;
2) que las mayores infecciones se verifican, cuando el trigo germina en un suelo que se va desecando progresivamente, por faltar el aporte de nuevas lluvias.

CUADRO 4.—(*Continuación*)

Variedad	Naturaleza	Clasificacion	Total espigas	Espigas "cariadas"	Porcentajes
Pelon Colorado	Sel. Masal	"	271	184	67.9
"	"	C.D.I.P.	271	184	67.9
K–08–26	L. Pura	C.D.I.	340	174	51.2
"	"	"	220	108	49.0
K–02–26	"	"	356	105	29.5
"	"	"	311	70	22.6
K–016–26	"	"	423	53	12.5
"	"	"	417	22	5.3
K–012–26	"	"	383	14	3.6
"	"	"	298	5	1.7
K–09–26	"	"	439	45	10.2
"	"	"	329	38	11.5
K–04–26	"	"	361	20	5.5
K–03–26	"	"	396	12	3.0
K–010–26	"	"	416	59	14.2
B x R–C	L. Pura H.	C.SMD.IP.	382	228	59.7
Alaska	Sel. Masal	B.B.P.	99	56	56.5
Pagador	L. Pura H.	C.SMD.IP.	161	76	47.2
Kota	Sel. Masal	C.SMD.I.	170	155	91.1
K–01–28	L. Pura	C.D.I.	355	4	1.1
Kanred	"	"	322	106	32.9

CUADRO 5.—*Con la lista clasificada de las variedades y porcentajes de infección en el lote No. 2, de las siembras de Abril 30 de 1928*

Variedad	Naturaleza	Clasificacion	Total espigas	Espigas "cariadas"	Porcentajes
Florence	L. Pura H.	B.SMD.P.	125	17	13.6
"	"	"	114	7	6.1
"	"	"	138	5	3.6
Tuzela	Sel. Masal	C.SMD.IP.	109	100	91.8
"	"	"	127	118	93.0
Székács 1055	L. Pura H.	C.D.I.	116	5	4.3
(B x R–C) x Kanred Sel. 2	"	"	121	54	44.7
" " 3	"	"	199	109	54.7
" " 4	"	"	194	135	69.5
" " 5	"	"	139	120	86.9
" " 10	"	"	187	101	54.0
" " 15	"	"	194	171	88.1
" " 20	"	"	173	131	75.7
" " 21	"	"	128	84	65.6
" " 22	"	"	112	56	50.0
Székács 266	"	"	100	11	11.0
" 304	"	"	104	9	8.6
" 319	"	"	137	116	84.6

Asi tenemos p. e., que el trigo Florence sembrado en Abril, recibiendo durante la germinación, 6 mm. de agua, dió una infección de 7.2 por ciento como promedio de 6 repeticiones y sembrado en Mayo, sin lluvias durante la germinación, dió una infección de 17.5 por ciento como promedio de 3 repeticiones (ver cuadros 4, 5 y 6).

CUADRO 6.—*Con la lista clasificada de las variedades y porcentajes de infección en el lote No. 1, de las siembras de Mayo 5 de 1928*

Variedad	Naturaleza	Clasificacion	Total espigas	Espigas "cariadas"	Porcentajes
Florence	L. Pura H.	B.SMD.P	285	61	21.5
"	"	"	261	45	17.3
"	"	"	379	52	13.7
"117" S 1	"	C.SMD.IP	180	100	55.5
"117" S 2	"	"	172	127	73.8
"117" S 5	"	"	177	85	48.1
"117" S 7	"	"	106	65	61.4
"117" S 8	"	"	185	62	33.5
Fuliginoso	Sel. Masal	C.SMD.P	79	10	12.7
Belosiornaia	"	B.SMD.IP.	315	276	87.6
Sary Maghis	"	B.SMD.IP.	159	137	86.1
Ulka	"	C.D.I.	283	157	55.4
Tchersnouska	"	B.D.P.	72	17	23.7
Tchersnoostaia	"	"	132	4	3.3
Saratov 41082	"	C.D.IP.	123	93	75.6
K–0279–26	L. Pura	C.D.I.	260	103	39.6
K–0375–IV–27	"	"	451	143	31.7
K–0375–V–27	"	"	380	69	18.1
K–0375–I–27	"	"	447	180	40.3
K–0375–II–27	"	"	283	98	34.6
K–0375–VI–27	"	"	282	118	41.9
K–042–I–27	"	"	311	104	33.5
K–042–II–27	"	"	460	157	34.2
K–042–V–27	"	"	357	166	46.5
K–04–5V–27	"	"	614	194	31.5
K–04–II–27	"	"	354	187	52.8
K–04–VII–27	"	"	338	169	50.0
K–04–III–27	"	"	321	223	69.5
K–04–I–27	"	"	396	229	57.9
K–04–V–27	"	"	367	211	57.5
K–04–VI–27	"	"	367	250	68.1
K–04–26	"	"	491	234	47.7
K–040–II–27	"	"	375	107	28.6
K–040–I–27	"	"	437	77	17.6
K–060–I–27	"	"	452	116	25.6
K–038–26	"	"	426	344	80.2
Kansas 5	"	"	292	34	11.7
" 4	"	"	343	82	24.0
Ballod 5	"	C.B.IP.	280	261	93.3
" 3	"	"	338	311	92.1
K–02–28	"	C.D.I.	305	71	23.3
Tuzela	Sel. Masal	C.SMD.IP.	280	274	97.7
103/16–B3 DG.	L. Pura	"	201	137	68.2
313 x B77 D.G.	"	"	196	177	90.4
X 51 D.G.	"	"	46	45	97.9
III a 2620 D.G.	"	C.SMD.P.	97	92	94.9
XIII a i D.G.	"	"	—	—	—

CUADRO 7.—*Con la lista clasificada de las variedades y porcentajes de infección en las siembras del lote No. 1, de Julio 17 de 1928*

Variedad	Naturaleza	Clasificacion	Total espigas	Espigas "cariadas"	Porcentajes
Barletta Campeon	Sel. Masal	C.SMD.P.	433	367	84.7
Red Bobs	L. Pura H.	"	358	237	66.3
Universal I	L. Pura	"	454	324	71.3
Record Sel. 1928	L. Pura H.	"	423	343	81.2
"38" M.A. Back. Dev.	"	"	182	78	43.0
"110" M.A. Back. Dev.	"	"	302	213	70.5
IV c 3 (17) O Klein	"	"	202	56	27.8
XIII t 1 Klein	"	"	375	63	16.8
H 26 h Klein	"	"	397	85	21.5
H 51 Klein	"	"	282	107	38.0
IV c 390½ Klein	"	"	238	104	43.7
H 28 d Klein	"	"	455	167	36.8
H 52 Klein	"	"	437	46	10.6
III a 123 IFLE	"	"	365	313	85.7
III a 21 (m III, IV) IFLE	"	"	352	281	79.9
IV c (1002, 1006, 1007) IFLE	"	"	235	94	40.0
1149 IFLE	"	"	229	109	47.6
III a 23 (m II) IFLE	"	"	399	329	82.5
III a 23 (m III)		"	363	288	79.4
IV c 100 IFLE	"	"	285	49	17.2
III a 12 IFLE	"	"	376	337	89.7
A c d 3 IFLE	"	B.D.P.	415	38	9.2
III a 23 (m y) IFLE	"	C.SMD.P.	358	331	92.5
IV c 1001, 1004 IFLE	"	"	79	41	51.9
III a 21 (m II) IFLE	"	"	266	222	83.4
IV c 1003111 IFLE	"	"	113	38	33.7
45 b IFLE	"	B.D.P.	211	2	0.95
IV y IFLE	L. Pura	"	209	107	51.4
III a 2620 IFLE	L. Pura H.	C.SMD.P.	297	273	91.9
313 x B77 $\frac{71}{25}$ B	"	"	335	268	80.3
313 x B77 $\frac{61}{25}$ G	"	"	358	271	75.7
XIII e i D.G.	"	"	247	145	58.7
XIII a i D.G.	"	"	143	118	82.6
313 x Bless 62 A D.G.	"	"	423	316	74.8
X 51 D.G.	"	"	252	180	71.5
Record 1925	"	"	329	283	86.0
Chileno	Sel. Masal	B.D.P.	113	24	21.3
Chargarod	"	"	134	17	12.7

CUADRO 8.—*Con la lista clasificada de las variedades y porcentajes de infección, en las siembras del lote No. 2, de Julio 17 de 1928*

Variedad			Naturaleza	Clasificacion	Total espigas	Espigas "caradas"	Porcentajes
Kanred × Universal II		148 D.G.	L. Pura H.	C.SMD.IP	406	38	9.35
"	"	188 "	"	"	501	412	82.2
"	"	166 "	"	"	507	76	14.9
"	"	134 "	"	"	580	98	16.9
"	"	143 "	"	"	384	82	21.4
"	"	133 "	"	"	526	147	28.0
"	"	130 "	"	"	740	310	41.9
"	"	137 "	"	"	665	186	27.9
Kanred 110 F. Pico D.G.			L. Pura	"	338	57	16.9
89/26–85 D.G.		"	"	"	455	372	81.7
Vencedor × Lin Calel		161 "	L. Pura H.	"	630	374	59.4
"	"	153 "	"	"	637	263	41.4
"	"	157 "	"	"	624	107	17.1
"	"	160 "	"	"	708	186	26.3
"	"	172 "	"	"	623	512	82.2
"	"	179 "	"	"	707	421	59.6
"	"	184 "	"	"	738	576	78.0
Vencedor × Kanred		185 "	"	"	737	337	45.7
"	"	185/A D.G.	"	"	618	30	4.9
"	"	203 D.G.	"	"	779	167	21.4
"	"	194/A D.G.	"	"	786	351	44.7
"	"	198 D.G.	"	"	596	144	24.1
"	"	196 "	"	"	658	337	51.3
"	"	187 "	"	"	585	214	36.5
"	"	194 "	"	"	695	413	59.4
80 C 17 158 Hib. Natural.		D.G.	"	"	746	128	17.2
80 C 10–3–172	"	"	"	"	559	419	75.0
183 C 146/25 Kansas		"	L. Pura	"	667	1	0.15
75/26–86 Kansas		"	"	"	727	2	0.27
170–147/25 Ruso		"	"	"	430	74	17.3

Abreviaturas:

C.D.I.	colorado, duro de Invierno.
C.D.P.	" " Primavera.
C.SMD.I.	" semidura de Invierno.
C.SMD.P.	" " Primavera.
C.SMD.IP.	" " Invierno y Primavera.
B.SMD.P.	blanco, semiduro de Primavera.
B.B.P.	" blando "
L. Pura	linea pura de "popblación."
L. Pura H.	" " "hibrido."
Sel. Masal	Seleccion Masal.
IFLE	Instituto Fitoténico de La Estanzuela.
D.G.	División de Genética.
M.A.	Ministerio de Agricultura de la Nación.
K.	Kansas.

Un caso notablemente confirmativo de esta conclusión, lo hemos tenido el ultimo año. En efecto, resultó que dado el fracaso de un ensayo de anticriptogámicos, que sembrado en Abril—a pleno campo no llegó a germinar por la sequia extraordinaria que sufrimos en 1929, repetimos éste ensayo en otro lugar, sembrando en Julio, en tierra tambien muy seca, que fué necesario regar continuamente hasta lograr una germinación pareja en todas las parcelas. Cosechados los ensayos oportunamente, nos encontramos con que el Testigo Infectado, solo daba una infección de 8.4 por ciento sobre 395 plantas bajo control.

La variedad usada en estas pruebas, es la Record Klein S. V. 1925, variedad cuya gran susceptibilidad a las infecciones experimentales, tal como nosotros las realizamos, la hemos establecido en repetidas ocasiones. Asi tenemos que el Record Klein, el año 1928, en cultivos comunes—no irrigados—nos dió los siguientes guarismos:

Record S. V.	1928	81.2	por ciento
" "	1925	86.0	" "
" "	"	86.4	" "
Promedio		84.5	" "

Todavia más. Una serie de 8 descendencias de la linea genética, III a o Artigas, el homólogo uruguayo de nuestro Record, nos da los siguientes valores:

DESCENDENCIA DE III, A.		
83.4	por ciento	
85.7	" "	
79.9	" "	
82.5	" "	(ver cuadro 7)
79.4	" "	
89.7	" "	
92.5	" "	
91.9	" "	
Pr. 85.6	" "	

De manera que no cabe dudar de la gran susceptibilidad fisiológica del Record Klein, a la caries.

Y entonces como interpretar su comportamiento en 1929, que lo incluiria entre las variedades fisiologicamente muy resistentes, sino tuviéramos los antecedentes que citamos?

Como la casi totalidad de las condiciones experimentales, han permanecido constantes en los ensayos de 1928 y 1929, es indudable entonces, la influencia decisiva del agua de riego, que le hemos prodigado durante la germinación, la que actuando como lluvias de poco milimetraje pero casi

diarias, han creado el ambiente desfavorable para la infección, que ya otros autores le reconocen, a las tierras saturadas de agua por lluvias repetidas: Boerger (3), Woolman y Humphrey (54).

(II) *Comportamiento según los caracteres filogenéticos de los grupos o subespecies.* En el cuadro 2, de este trabajo, exponemos la lista de las subespecies que hemos ensayado, clasificadas según sus caracteres genéticos y morfológicos, conforme a la clasificacion de Vavilov.

En el cuadro 9 que acompañamos, hemos agrupado ciertas variedades de *Triticum vulgare* y todas las demás de las otras subespecies, a los efectos de facilitar una visión rápida del conjunto.

El cotejo de los diversos promedios, permite llegar a las siguientes conclusiones:

1) que de un modo general, los trigos a 14 pares de cromosomas, presentan mayor resistencia media que los trigos a 21 pares de cromosomas (con excepción del grupo de los "hard red winter" confirmando las observaciones de Vavilov (51), Percival (38), Tisdale (48) y otros autores.
2) que la subespecie *Triticum vulgare,* presenta la variabilidod mas extraordinaria en lo que respecta al comportamiento de sus variedades a la infección por Tilletia, siendo interesante constatar que Tapke (46) llega al mismo resultado, en sus últimas investigaciones sobre el carbón volador (*U. tritici*).

Debemos destacar que la primera conclusión, no tiene valor de generalización en nuestros ensayos, pues solamente hemos testado, ocho variedades a 14 pares de cromosomas, las que dán un promedio general de 17.5 por ciento, con valores limites de 0.9 a 56.5 por ciento.

Es probable que trabajando con un gran número de variedades y con diversas colecciones de Tilletias, se encuentre en este grupo tanta variabilidad en la resistencia, como en el grupo de los *Triticum vulgare.*

Pese a que autores tan eminentes como Vavilov, Percival, Sax y Stakman, han creido reconocer en este grupo, una resistencia general a las enfermedades, que vinculaban a su peculiar estructura genética, dia a dia esta conclusión ha ido perdiendo su consistencia, en virtud de las nuevas experiencias realizadas. Limitándonos a lo referente a las Tilletias, ya en el trabajo relatado por Tisdale (l. c.), puede verse que ciertos *T. durum* son susceptibles de infecciones pesadisimas, (hasta 92.7 por ciento en la variedad Marouani C. I. 2235), con la colección de *T. tritici* usada en Davis (California).

Brentzel y Smith (4), en experimentos conducidos en Fargo y Dickinson (North Dakota), encuentran que allí, los *Triticum durum,* son un 100 por ciento mas susceptibles a la *Tilletia tritici* que a la *T. levis,* mientras que los "hard red spring wheats," presentan la reacción opuesta.

CUADRO 9.—*Mostrando la susceptibilidad a la caries, según los caracteres filogenéticos de las subespecies y su hábito de vida.*

Triticum vulgare

Variedades de Invierno		Variedades de Primavera	
Lin Calel	94.3 por ciento	Barletta	84.7 por ciento
Kanred	32.9 " "	Universal I	71.3 " "
Ruso mútico	95.5 " "	Record 1928	81.2 " "
Ruso aristado	97.1 " "	"38" M.A.	43.0 " "
Pelón colorado	76.3 " "	"110" M.A.	70.5 " "
Tuzela	92.4 " "	Promedio	70.1 por ciento
Kansas	18.4 " "		
Williamson	74.3 " "		
Promedio	72.6 por ciento		
Excluyendo Kanred y Kansas, el promedio asciende a	83.3 por ciento		

Nuevas variedades de Invierno		Nuevas variedades de Primavera	
K–016–26, Cheg	8.9 por ciento	XIII t 1 Klein	16.8 por ciento
K–012–26, "	2.6 " "	H 26 h "	21.5 " "
K– 09–26, "	10.8 " "	IV c 390½ "	43.7 " "
K– 03–26, "	3.0 " "	H 28 d "	36.8 " "
K– 01–28, "	1.1 " "	H 52 "	10.6 " "
Székács 1055	4.3 " "	H 51 "	38.0 " "
" 266	11.0 " "	Promedio	27.9 por ciento
" 304	8.6 " "		
146–183– c D.G.	0.15 " "		
75/26–86 D.G.	0.27 " "		
Promedio	5.07 por ciento		

Triticum compactum

Ballod 5, Cheg	93.3 por ciento
" 3, "	92.1 " "
Promedio	92.7 por ciento

Triticum durum Desf.

Tchersnouska	23.7 por ciento
Tchernoostaia	3.0 " "
A c d 3 IFLE	9.2 " "
45 b "	0.9 " "
Chileno	21.3 " "
Chargarod	12.7 " "

Triticum persicum Vav.

Fuliginosum	12.7 por ciento

Triticum turgidum L.

Alaska	56.5 por ciento

Reichert (l. c), encuentra que en Palestina, los *Triticum durum* son normalmente más atacados por la Tilletia de esa region que los *Triticum vulgare,* lo que hace presumir la existencia de formas fisiológicas especialmente virulentas sobre los *T. durum.*

Recientemente Holton (l. c.), ha demostrado que una colección de *Tilletia tritici* de Devils Lake (North Dakota), era extremandamente virulenta sobre los *Triticum durum,* comunmente cultivados en esa región, mientras que ciertas variedades a 21 pares de cromosomas como el Marquis, Marquillo, y Hope, permanecian practicamente inmunes. Indudablemente lo que ha complicado notablemente estas investigaciones, ha sido la existencia de formas fisiológicas especializadas en ambas Tilletias.

Probablemente en un futuro proximo, nuevas investigaciones debilitarán cada véz más las conclusiones de Vavilov (l. 51), sobre interrelaciones, de la inmunidad con la naturaleza filogenética de las subespecies, a las que no obstante debe reconocerse el servicio inapreciable que han prestado a la investigación, como "hipótesis de trabajo."

(III) *Comportamiento de los Triticum vulgare.* En primer término, lo que llama la atención, es la variabilidad extraordinaria, de la resistencia de sus variedades y "pequeñas especies" (lineas puras en el concepto de Johannsen).

Se tiene toda la gama, desde la enorme susceptibilidad revelada por un Ruso aristado, Tuzela o Lin Calel, con porcentajes de 100 por ciento de infección "en plantas" y 95 a 98 por ciento "en espigas," a la resistencia rayana en la inmunidad, de las nuevas lineas genéticas 146–183 c D. G.; K–01–28 Cheg; etc., con valores hasta de 0.15 por ciento.

Por otra parte interesa constatar, que el mayor número de "formas nuevas muy resistentes" se encontraron en un sub-grupo de trigos aristados, a espiga laxa y blanca, de hábito invernal y especialmente adaptados a vivir en regiones frias y secas, (*Triticum vulgare* v. *erythrospermum*).

Este subgrupo esta constituido por trigos de invierno de Hungria (Székács Winter Weizen), lineas puras de Kansas de nuestra experimentación y lineas puras de Kansas de la División de Genética Vegetal.

Es conveniente hacer notar que dichas lineas puras de Kansas, proceden de un material ensayado por Backhouse (2), en 1916, que se trataba sin duda, de algunas de las selecciones que según Jardine, (27) fueron aisladas por H. F. Roberts, del Crimean C. I. 1435, que dió como resultado la creación del Kanred (ex- P–762), (6).

Dicho Crimean C. I. 1435, es un trigo colorado de Invierno y según Clark (6), fué importado a Estados Unidos, en 1900, procedente de Ambracievka, Territorio del Don (Rusia). (De un punto muy próximo a Ambracievka seria oriundo el Turkey, variedad muy difundida en Estados

Unidos, que en las pruebas de Gaines (17), dió solo 1.8 por ciento de infección por *Tilletia tritici*).

Es interesante referirnos al común y primitivo origen geográfico del Kanred y nuestras lineas puras, por cuanto además de su resistencia al frio y a la seca, una común resistencia a las enfermedades las identifica.

Ya nos hemos referido a la inmunidad del Kanred con respecto a 11 formas fisiológicas de *Puccinia graminis tritici* Eriks. and Henn. (32), y agregaremos que Kiesselbach y Peltier (29), estudiando 578 selecciones de Crimean C. I. 1435, encontraron un grupo de selecciones mas resistentes que el Kanred o inmunes a nuevas formas fisiológicas a las que el Kanred era susceptible.

Ya hemos visto en el cuadro 9, como se comportan algunas de nuestras lineas muy resistentes, y la enorme diferenciación fisiológica que revelan al cotejarlas con otras estirpes genéticas regionales, tales como el Lin Calel M. A., o el Williamson.

No se crea que todas las lineas de Kansas, son tan resistentes como las mencionadas. Dentro de una resistencia próxima a la del Kanred (30 a 40 por ciento), se agrupa la mayoria de las lineas restantes, presentándose como una excepción la linea K–038–26, Cheg con una infección de 80.2 por ciento.

Debe llamarnos la atención que casi todas estas variedades muy resistentes, pertenezcan a un mismo tipo biológico (*Triticum vulgare* v. *erythrospermum*), y que muchas de ellas tengan la misma cuna geográfica. (No debe olvidarse sin embargo que tipos muy resistentes, tambien los hay en *T. vulgare* v. *albidum* ej. Florence; en *T. vulgare* v. *ferrugineum* ej. K–09–26 Cheg.—Una linea de *T. vulgare* v. *milturum,* la M–0274 de la Estacion Experimental de Odessa, tambien ha sido señalada como inmune por Talanoff (45). Una estirpe fisiologicamente inmune puede citarse para *T. compactum* v. *humboldtii,* merced al nuevo hibrido de Gaines, el Albit C. I. 8275 (Hibrido 128 x White Odessa), (7 y 18).

Anteriormente hemos afirmado que en las regiones frias y secas, como el S. E. de la Pampa, se tendrian las condiciones mas favorables para la producción de fuertes infecciones, confirmando nuestra conclusión, observaciones analogas de Tingey (l. c.) en Nebraska, Dickson (l. c.) en Kansas, y Tisdale et al. (l. c.), en los estados norte americanos de la costa del Pacifico.

Es indudable que la Crimea rusa tiene grandes similitudes agrológicas y climatéricas con el Oeste de Kansas y La Pampa—buena prueba de ello, es que sus tipos biológicos se adaptan perfectamente a estas ultimas regiones —y que las condiciones del ambiente son muy favorables a la infección por caries.

Como entre sus tipos indígenas de trigo, pueden aislarse estirpes altamente resistentes, la lógica nos llevaria a enunciar la siguiente conclusión:

> que cuanto más favorables a la infección son las condiciones climatéricas y agrológicas de una región, mayores son las probabilidades de encontrar entre las viejas razas autóctonas, estirpes altamente resistentes.

Esta conclusión seria concorde con las ideas de Nilsson-Ehle (37), sobre la adaptación.

(IV) *Comportamiento del T. persicum.* Por ser una subespecie poco conocida nos detendremos brevemente, consignando los datos mas recientes y autorizados sobre la misma.

Es una nueva especie establecida hace pocos años por Vavilov (51). Zhukowsky (55), y Atabekova (1), han clasificado numerosas variedades de esta "nov. sp.," bien diferenciadas por los caracteres de su espiga, caracteres vegetativos y biológicos.

Percival (l. c.), la habia considerado una variedad de *Triticum dicoccum* y anteriormente habia sido descripta como *T. vulgare* v. *fuliginosum.* Morfologicamente es muy semejante al *T. vulgare,* pero por sus caracteres fisiológicos y número de cromosomas es un *T. durum.*

Fundado en estas caracteristicas, en su cuna geográfica y centro de diversidad (9), Vavilov (52), sostiene que se trata de un hibrido inter-especifico de *T. vulgare* x *T. durum* y representaria una forma de transición.

Inmune al "Oidium" (*Erysiphe graminis*), Vavilov (50) establece esta caracteristica como una caso único entre otras 580 variedades por él cotejadas.

Susceptible a la *Puccinia triticina,* ha sido cruzada por Vavilov (50) en Reading (Inglaterra), con una variedad de *T. vulgare* inmune a dicha "roya," obteniendo en la F_1 o primera generación, un hibrido inmune a ambos parásitos, probando la dominancia de la inmunidad como caracter hereditario.

Vavilov (51), lo encuentra muy susceptible a la caries en pruebas que realiza al efecto, pero agrega que un proceso de incompatibilidad puede observarse, ya que los granos "cariados," son pequeños y abortivos.

En nuestras pruebas se reveló como muy resistente a la *Tilletia levis,* dando solo 12.7 por ciento de infección.

Según el mismo autor, es facilmente cruzado con los *T. durum,* con los que daria hibridos perfectamente fértiles. Segun Percival (38) con los *T. vulgare,* daria hibridos "casi estériles" en la F_1.

Nuestras observaciones sobre hibridos inter-especificos (33), nos dán con respecto al *T. persicum* v. *fuliginosum,* los siguientes resultados:

en el hibrido *T. vulgare* v. *ferrugineum* x *T. persicum* v. *fuliginosum,*

sobre 16 flores fecundadas artificialmente, obtenemos 12 granos bastante "chuzos," lo que revelaba ya, un proceso de incompatibilidad genética.

de los 12 granos nacieron 9 plantas que se desarrollaron bien, siendo perfectamente visible en todas, su naturaleza hibrida, por la dominancia de caracteres tales como, Pubescencia de las Glumas, Hollin o "fuligo" de las espigas, Bordes colorados de las Glumas, etc., todos caracteres distintivos del ascendiente paterno;

esta F_1 presenta basteantes pústulas de *P. triticina, P. glumarum* Eriks. and Henn. y *P. graminis tritici,* mientras que el *T. persicum* se manifestó casi inmune a la *P. graminis tritici* y muy poco atacado por las *P. triticina* (Schm.) Eriks. & Henn.; y *P. glumarum;*

como el ascendiente susceptible—el *T. vulgare* v. *ferrugineum*—se manifestó a la véz extraordinariamente atacado por *P. glumarum* y *P. graminis tritici,* tendriamos una nueva prueba confirmativa de la naturaleza recesiva del caracter "resistencia a las royas" (32);

finalmente examinando las espigas hemos constatado la casi total autoesterilidad, del hibrido en F_1;

en una planta, sobre 221 flores solo fecundaron y formaron granos, 11;

en una segunda sobre 402 flores, se obtuvieron solamente 24 granos; es decir un 95 por ciento y un 94 por ciento respectivamente de "autoesterilidad";

los granos son extremadamente "chuzos" y ahora falta saber en que proporción seran "autovitales."

(V) *Regularidades observadas en la resistencia a diversas enfermedales.* Es interesante hacer notas que Tapke en un valioso trabojo sobre el carbón volador (*Ustilago tritici*), llega a conclusiones semejantes a las que hemos enunciado.

Dice este autor en su "Occurrence of loose smut on wheat" (46):

Pentad, el unico *Triticum durum,* incluido en los ensayos, fue altamente resistente (0.20 por ciento). Los tres trigos "club" cotejados (*T. compactum*), se mostraron fuertemente susceptibles, alcanzando hasta un 97 por ciento de espigas atacadas en el Little Club C. I. 4066. En los trigos comunes (*T. vulgare*), la escala de resistencia vá desde la mas elevada susceptibilidad, a la inmunidad absoluta. Las variedades altamente resistentes son relativamente pocas, pero una o mas se encontraron en cada uno de los cuatro grupos comerciales, excepción hecha de los trigos blancos (*T. vulgare* v. *albidum; T. vulgare* v. *graecum,* etc.).

en trigos "poblaciones" tales como el Fulcaster, Gipsy, Harvest

en trigos "poblaciones" tales como el Fulcaster, Gipsy, Harvest

Queen y Red May.

Las lineas puras de Hussar (Red Hussar C. I. 4843) y Ridit (Smut-proof C. I. 6703), son altamente resistentes e immune respectivamente al "carbón volador."

Estas mismas selecciones han demostrado ser inmunes a la caries.

Fué evidente la existencia de formas fisiológicas especializadas, en *U. tritici.*

No se encontró correlación entre los valores "iones-hidrógeno" de los jugos (acidez) y la capacidad de resistencia de los huéspedes a la infección.

Y es interesante, repetimos, por cuanto las similitudes observadas en el comportamiento a diversas enfermedades, tales como Puccinias, Tilletias, Ustilago, etc., cuando se consideran diversos grupos filogenéticos, permiten sospechar la existencia de condiciones generales que regularian las infecciones, tales como la Naturaleza Genetica De Las Subespecies, problema especialmente estudiado por Vavilov y que fundamenta una de sus leyes sobre la "Distribucion de la Inmunidad en las Plantas" (51). O la elevada Actividad Osmotica Endocelular, argumento sostenido entusiastamente por Draghetti (13), quien vincula la elevada resistencia de los *T. durum; T. vulgare* v. *esythrospermum,* etc., a que la subespecie o variedad botánica considerada, presentaria comunmente el Máximo Número y Las Máximas Manifestaciones De Los Caracteres Hipertonizantes De Los Jugos. Hoy, ya no se acepta, que la resistencia a las enfermedades deba referirse a la cantidad de ácidos orgánicos del contenido celular, hipótesis sostenida por Comes, Lo Priore y Kirchner y otros autores, Negada esta teoria por los trabajos de Vavilov, es nuevamente desautorizada por las observaciones recientes de Hurd-Karrer y Tapke (24, 25 y 46).

Es indudable que ya no puede aceptarse ninguna hipótesis simplista sobre la naturaleza de la resistencia a las enfermedades y que limitando a sus verdaderas proporciones, los casos de Resistencia Morfológica, reconocidos por Hursh (26) y de Resistencia Funcional reconocidos por Hart (21), debe admitirse la compleja naturaleza de la Resistencia Fisiológica, la que al decir de Vavilov, seria la resultante de muy complicadas interrelaciones fisiológicas entre el parásito y el protoplasma de las células huéspedes.

(VI) *Irregularidades que se observan cuando se comparan los resultados obtenidos en diversos lugares.* Por lo general, cuando se comparan los resultados de ensayos de infección, realizados en diversas situaciones geográficas, se observa que los mismos difieren casi completamente.

Estas diferencias lejos de ser anormales, responden a causas naturales, cuya verdadera naturaleza conviene siempre establecer. Asi, nuestros resultados, obtenidos en Guatraché, en el S. E. de La Pampa, difieren mar-

cadamente de los de Brunini, obtenidos en Barrow, en el S. E. de Buenos Aires.

Concretando lo que hemos expuesto en otro lugar (34), la no coincidencia de los resultados entre dos experimentaciones, tales como la de Guatraché y Barrow, puede imputarse a algunas de las siguientes causas:

1) Influencia del ambiente (temperatura, humedad y suelo).
2) Influencia de la época de siembra.
3) Naturaleza especifica del parásito; posible existencia de formas fisiológicas especializadas, (sobre este particular conviene recordar que han sido reconocidas recientemente, numerosas formas fisiológicas especializadas en ambas Tilletias, por Faris (14), Rodenhiser y Stakman (41), Reed (39), Rodenhiser (42), Gaines y Romer (19), Gaines y Smith (20), Reichert (40), Holton (23); etc. Reed, solamente, en 42 colecciones testadas sobre 18 huéspedes diferenciales, prueba que hay por lo menos 4 f. f. en *Tilletia levis* y 6 f. f. en *T. tritici,* en sus colecciones).
4) Errores en la identificación de las variedades.
5) Empleo de distintas lineas genéticas (de una variedad común), con distinta susceptibilidad.
6) Naturaleza de la infección experimental. Métodos de investigación y exposición de los resultados.
7) Causas accidentales no establecidas o no sospechadas por el investigador.

SUMMARY

Object and Methods.—Tilletia levis and *T. tritici* have been found in different sections of the Argentine wheat area, but the geographic distribution, comparative virulence, and forms of each are not yet exactly known.

Particularly in the winter-wheat area, which embraces La Pampa and Western and Southern Buenos Aires, bunt annually produces considerable loss.

Although there has been an increasing development of seed-disinfection methods involving use of chemical dusts, such as copper carbonate, Uspulun, Abavit 26, and others, many farmers still employ copper sulphate, which often produces severe losses through seed injury.

In some sections, also, infection occur through bunt spores present in the soil.

The virtual impossibility of an effective control of this disease in regions subject to soil infestation has impelled the Dirección General de Enseñanza y Fomento Agricola del Ministerio de Agricultura de la Nación, to develop for such sections varieties of wheat inmune from *Tilletia,* spp., varieties that are at the same time acceptable agronomically and industrially. This

work will be carried out by the Guatrache Experiment Station. A preliminary study of varietal resistance of wheat to *Tilletia levis* collected in Guatrache was made involving 154 varieties belonging to 5 subspecies. Of the 154 varieties 80 were winter wheats and 74, spring or midseason varieties, comprising most of the wheats cultivated in Argentine, new pure lines and hybrid selections still in the nursery tests, and some well-known foreign varieties.

The seed was heavily inoculated just before seeding with fresh spores of *Tilletia levis* obtained the previous year from the variety Tuzela. The seed was sown on three consecutive dates, April 30, May 5, and July 17; the temperature and humidity conditions were carefully noted during the period of germination following each date. The results are given in percentages of bunted heads, being based on the examination of 59,194 heads of this crop. They refer only to the experiments of 1928. The same varietal trials repeated in 1929 were destroyed by an unusual drought that killed all the crops in this year.

The desire to prevent any delay in the presentation of the results of these experiments, the first ever obtained on a large scale in Argentine, justifies their presentation at this time.

Conclusion: 1. The most favorable conditions for infection of fall-sown winter varieties by Tilletia obtained in April and May.

2. The widest spread of infection was noticed where wheat germinated in a soil gradually drying out from lack of rain.

3. Generally, with the exception of varieties belonging to the hard red-winter group wheats of 14 pairs of chromosomes offer greater resistance to bunt than do those of 21 pairs.

The writer, however, believes after treating a larger number of varieties of 14 pairs of chromosomes with different collections of Tilletia, almost as great a variation in resistance should be encountered as that observed in the *T. vulgare* group.

4. The subspecies *T. vulgare* presents the most extraordinary variability in respect to the behavior of its varieties to infection by Tilletia, ranging from almost total susceptibility to almost complete immunity. It is of interest to note that most of the new very resistant strains are found in a subgroup of winter-habit wheats, with bearded, lax, white heads, and hard red kernels that are specially adapted to cold, dry regions (*T. vulgare* v. *erythrospermum*).

5. Very susceptible to highly resistant strains have been found in a local variety known as Kansas, which is probably the same as Crimean C. I. 1435 of the United States Department of Agriculture.

Three years of yield experiments with these new strains indicate a possibility of their becoming valuable commercial varieties.

6. Certain strains of winter wheats from Hungary and the variety Florence from Australia were shown to be highly resistant.

7. All the highly resistant strains have been used to cross with other highly productive Argentine varieties of good milling and baking qualities.

ESTACION EXPERIMENTAL DE GUATRACHE (PAMPA CENTRAL),
MINISTERIO DE AGRICULTURA DE LA NACION,
REPUBLICA ARGENTINA.

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BORDEAUX MIXTURE AS A FACTOR INCREASING DROUTH INJURY[1]

J. D. Wilson and H. A. Runnels

Ginseng, *Panax quinquefolium* L., is commonly grown in wood lots in Ohio. Those best adapted to the culture of this crop consist of large trees in open stand, their branches just interlacing so that the shade provided is continuous and of a fairly uniform density. All underbrush is removed and the beds are then prepared, much as when lath shelters are used.

Many of the ginseng plantings in Ohio are frequently attacked by Alternaria blight (*Alternaria panax* Whetzel) and, for this reason, must be sprayed. The most common treatment is that involving three or four applications of a 3–3–50 Bordeaux mixture. The timing of the sprays is regulated by the growth stage of the plant, as follows: Just as the majority of the plants are through the ground, after the leaves are fully expanded, shortly before bloom, and, finally, just after the fruits are well formed. During the past four summers the writers have been treating several plots in each of two wood lots near Wooster, Ohio, in an effort to determine some of the most effective spray and dust mixtures for the control of the disease mentioned above.

In 1930 the first application of materials was made on May 15, the second on May 31, and the third on June 25, and the fourth was omitted. At the time of the third application a considerable number of plants in both wood lots were beginning to show a type of ginseng foliage injury with which neither the growers nor the writers were familiar and which was considerably different from that commonly caused by improperly prepared Bordeaux mixture.

The first indication of this injury was a drooping of the individual leaflets. Next, the tissue at the margins of the leaflets suddenly began to collapse, first taking on a darkened, water-soaked appearance, and, later, drying out. The silvery green of the ginseng leaflets faded somewhat but did not disappear, nor did the blades become yellow. The margins of the leaflets finally became wrinkled and crisp. The drying-out progressed from the tip toward the base and from the edges inward much like tipburn in such plants as the potato. The tissue adjacent to the midrib and near the base of the leaflet often remained alive and green for some time after the margins were dead and dry. The plant at this stage appeared much as in figure 1, A, which was photographed on June 25. This may be com-

[1] Published with the approval of the Director of the Ohio Agricultural Experiment Station, Wooster, Ohio.

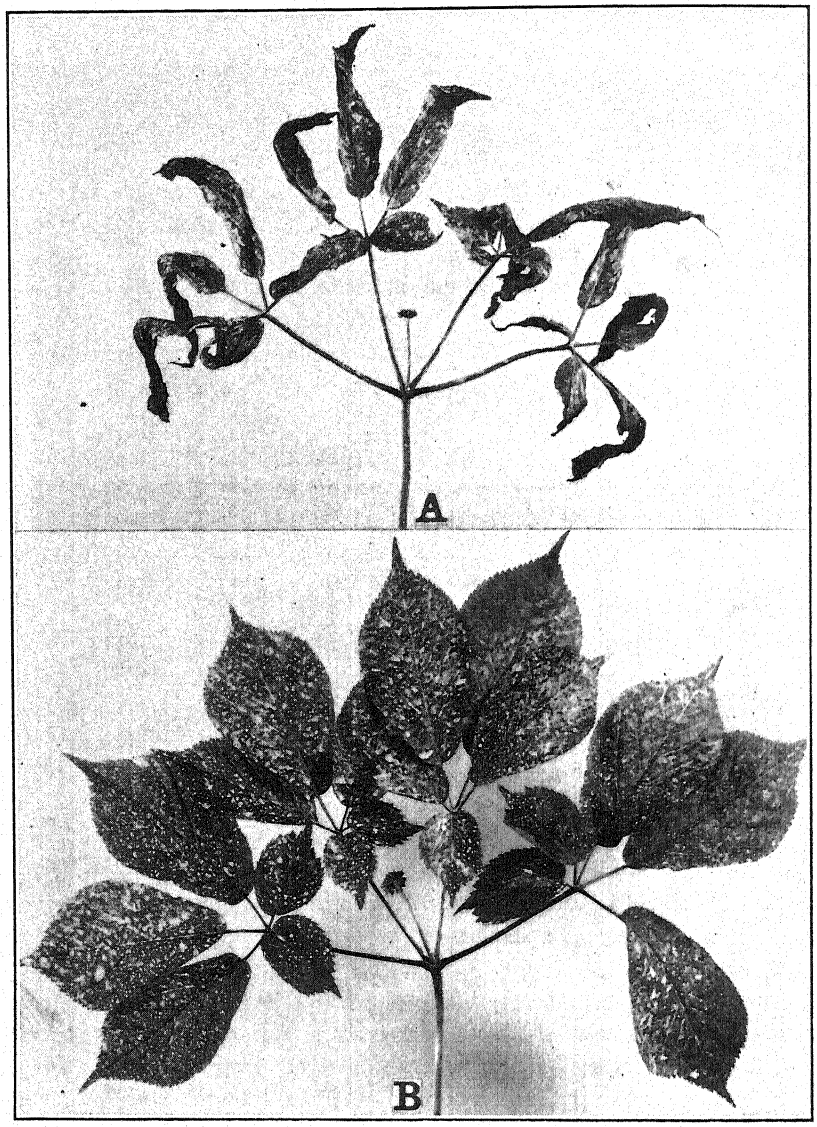


FIG. 1. A. Combination drouth and spray injury on ginseng. Sprayed with Bordeaux mixture. B. Normal ginseng plant sprayed with Bordeaux mixture.

pared with the normal plant of figure 1, B. Finally, the leaflets became tightly curled about the petioles, the latter then collapsing at their point of attachment to the stem. The leaflets remained attached to the petioles in many cases.

The injured-plant areas became progressively larger and new ones appeared as the days passed. By July 25 such a large proportion of the plants in both plantings had died that it was considered useless to make the fourth application of materials to the experimental plots. The majority of the plants failed to bloom properly and many of the young fruits dropped before maturity.

The general appearance of the dying plants and the manner in which the injury areas increased in size and number suggested drouth damage. The fact that many herbaceous plants in fields and wooded areas were beginning to suffer from a lack of soil moisture and the extremely dry aerial environmental conditions made it seem even more likely that the ginseng plants were being injured in the same way. Shade-loving plants, as a group, of which ginseng is a member, are particularly sensitive to conditions of high evaporation, lack of soil moisture, and an excess of light over the normal for their habitat. Burns (3) reported the death of pine seedlings, in exposed situations during a period of drying winds, and the marginal burning of leaves of other shaded plants when suddenly exposed to bright sunlight. Pool (18) noted that many species, normally occurring on forest floors, were either scarce or absent during a dry summer in Nebraska. Observations similar to those of Pool were made in Ohio forests during the summer of 1930. The competition for water finally became so severe that many of the trees began to lose some of their leaves and, by mid-July, many of the beech trees in Ohio had been defoliated.

The rainfall at Wooster from May 1 to July 31, 1930, inclusive, was only 51.4 per cent of the normal. The mean temperature was 2.4° F. above normal. The evaporating power of the air, measured with white, spherical atmometers, was 51.1 per cent higher than the average over the same period for the summers of 1928 and 1929. Drouth conditions during July, the month of greatest injury to vegetation, were especially severe. The rainfall was only 42.1 per cent of the normal and the evaporation total was 77 per cent greater than the average value for the same month in 1928 and 1929. This low rainfall and high evaporation rate resulted in the establishment of semixerophytic conditions under which many of the plant forms accustomed to a more plentiful moisture supply were not able to survive. Many species succumbed wholly or in part and many individual plants of different species lost all or part of their foliage. Thus it is not strange that a tender plant like ginseng should show injury, especially when in competition with deep-rooted trees for soil moisture.

On July 25, at the time it was decided not to put on the fourth spray application, there were still a number of isolated areas in each wood lot in which the plants were in good condition. Some of these areas were obviously in situations where the soil moisture content had remained sufficiently high to maintain plant growth, but a very striking thing was noticeable about the remainder, which comprised whole beds in which all of the plants appeared normally green. These beds were in the midst of other large areas in which all of the plants were dead, and they consisted of those plots which had not been sprayed at all during the season or had been treated with some compound other than Bordeaux mixture. With the exception of these few plots the entire area of each planting had been sprayed by the growers or the writers with three applications of a 3–4½–50 Bordeaux mixture (hydrated lime). In the check plot (nontreated) those plants that had not been affected by Alternaria blight were normal in color and form, as shown in figure 2, A, while those in immediately adjacent beds which had been sprayed with Bordeaux mixture were dry and dead, as in figure 2, B.

All the beds in which injury was noticeable as early as June 25 had received applications of Bordeaux mixture on May 15 and 31, respectively. This fact, connected with the observations made above, suggested the possibility that the mere presence of the Bordeaux residue on the ginseng leaves was in some way responsible for the injury starting as early as it did and becoming as severe as it finally was. That is, the Bordeaux must have exerted some accelerating influence on the transpiration rate of the plants. This increased transpiration rate, combined with the low soil moisture content and the high evaporating power of the air, placed a demand upon the water-absorption system of the plant which it was unable to meet. As a result the leaves passed through the successive stages of incipient drying, and transient and permanent wilting and, finally, under the influence of continuous conditions of desiccation they died and became dry.

The literature dealing with the effect of Bordeaux mixture upon plants is extensive and varied. In a discussion of the type of injury noted here on ginseng we are concerned chiefly with those papers which make reference to the water relations of the plant. For the purpose of discussion those which we shall consider may be roughly divided into five groups. The first group, which is not so directly pertinent as the rest, includes a few of the articles which discuss Bordeaux injury under certain types of weather conditions. The second includes those reporting a lowering of leaf temperatures; the third, a number which note a protective action against drouth; the fourth, those which indicate that transpiration rates are decreased; and, the fifth, those which show that the application of a Bordeaux spray to the leaves of a plant usually increases the rate of water loss.

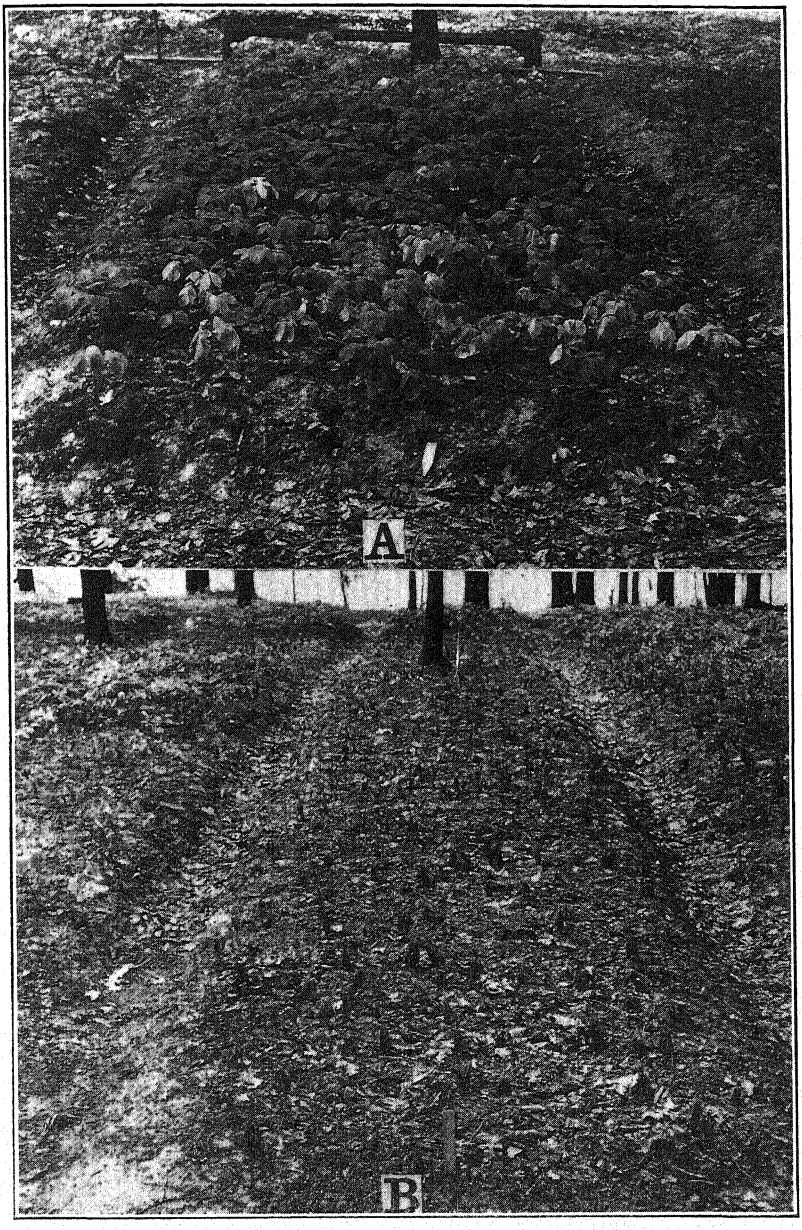


FIG. 2. A. Check plot. No spray. No drouth injury. B. Plot treated with 3–4½–50 Bordeaux mixture. Drouth injury.

Bain (1) found that peach trees sheltered from dew or rain or shaded were not affected by a Bordeaux spray which did injure those not so protected. Bonns (2) noted that wet weather following Bordeaux applications on apple trees increased leaf injury and that such factors as high atmospheric humidity and free water on the leaves were contributory. Crandall (6) reported Bordeaux injury as occurring regardless of the care used in the manufacture and application of the mixture if weather conditions, such as periods of rain or heavy dew formation, were just right following spraying. Hedrick (13) states that the kind of weather following an application is one of the factors determining the extent of Bordeaux injury and that alternating periods of rain and bright sunshine are most conducive to injury. Whetzel (23) reported injury to ginseng apparently due to the combination of a Bordeaux spray and cold weather. Many of the young plants were killed in this instance.

The type of injury made reference to in the above paragraph is most likely to occur when a spray application is followed by cool, wet weather or a series of heavy dews, possibly alternating with other periods of bright sunshine. The injury to ginseng in 1930 occurred during an extended period of dry, hot weather. Dew was not a factor since it did not form on these plants growing under trees. Also, the plants were shaded and rains were, of course, very infrequent. Thus, every factor cited as being contributory to the type of Bordeaux injury discussed in the previous paragraph was absent or opposite in occurrence, in the present case, and it therefore seems probable that the type and cause of the injury must have been quite different in the two instances.

Eaton and Belden (11), working with cotton, found that leaves covered with a coating of whitewash were about 3° C. cooler than nontreated checks. Lutman (14) found that a Bordeaux residue lowered the temperature of potato leaves from $\frac{1}{3}$ to $\frac{2}{3}$ of a degree Centigrade. Tilford and May (22) reported that a Bordeaux film lowered the internal temperature of a potato leaflet significantly below that of nonsprayed controls. An increase in the lime constituent further decreased the leaf temperature, while the addition of lampblack raised it to a point above that of the leaves on the control plants.

Lutman (15) found that the presence of a Bordeaux residue on potato plants afforded some degree of protection against that type of tipburn due to dry, hot weather, by virtue of its shading effect. Schander (20) also suggested a protection to plants from the same cause. Sturgis (21) regarded Bordeaux mixture as a beneficial factor, aiding plants to resist drouth injury during the drouth of 1894, in Connecticut, when the extreme heat and lack of soil moisture resulted in leaf burning of nonsprayed plants.

Clinton (5) attributed the increase in potato yields, which often results from the application of Bordeaux mixture in dry seasons, even in the absence of disease, to a decrease in the transpiration rate. Ewert (12) reported a decrease in transpiration rates following applications of Bordeaux; and Rumm (19), working with abscised stems of grape, found that those which had been sprayed with Bordeaux remained turgid longer than those not treated. Schander (20) also noted a lessening of transpiration due to spraying.

Bain (1) noted that peach seedlings, sprayed with Bordeaux, required watering more often than those not so treated. Butler (4) found that a coating of Bordeaux spray increased transpiration in a number of trials. Milk of lime also caused an appreciable increase. The more opaque the mixture the greater the acceleration observed. Most of the increase occurred during the night. Duggar and Cooley (7, 8), working with tomatoes, obtained an increase in the transpiration rate with Bordeaux films but not with a number of other sprays and dusts. Strong Bordeaux gave a greater acceleration than weaker mixtures. Some of the increases noted, as with Bordeaux mixture or this compound plus lampblack, were as great as 50 to 75 per cent over the control plants. High concentrations of Bordeaux induced incipient wilting and, even marginal leaf injury, in some instances. This effect was perhaps very similar to the ginseng injury observed in Ohio this year. Duggar and Bonns (9) in a study on potted potato plants found that most of the increase occurred during the night period. The increase ranged between 37 and 133 per cent over that of the controls. Increases as great as 479 per cent over the normal were noted for short intervals immediately following a spray application. Dutton and Wells (10) observed that cherries were often small on trees sprayed with Bordeaux mixture. Cherry shoots treated with Bordeaux lost water from 25 to 50 per cent faster than controls and the fruits became badly shrunken in a short time, thus indicating a removal of water from them by the rapidly transpiring leaves. Martin (16) applied Bordeaux mixture to abscised leaves and to potted plants and obtained a material increase in the rate of water loss. The increase was greatest during the first two hours after spraying. It also varied in degree for different species. Martin and Clark (17) sprayed potato plants growing in pots maintained at different soil-moisture contents with a 5–5–50 Bordeaux mixture. With high soil moisture the plants sprayed with Bordeaux lost 135 per cent more water than the controls during the night period and 81 per cent more during the day period. With low soil moisture the increases were 81 and 18 per cent, respectively. Zucker (24) found that Bordeaux increased transpiration on a variety of plants, while milk of lime was less effective in this respect.

It seems certain that the influence of the factors of low temperature, dew formation, and the alternation of rain and sunshine may be largely disregarded in conection with the type of injury noted in this instance. It is also very probable that no beneficial effect due to shading or decreased water loss existed since most of the plants were well shaded and only the sprayed ones showed injury. Thus, in view of the fact that a Bordeaux film on the leaves of a plant probably increases rather than decreases the transpiration rate and the further facts that the soil moisture content was low and the evaporation rate high, it seems very likely that the death of the sprayed plants was in some way brought about by the combined effect of drouth and spray which seriously disturbed the internal water relations of the plants. This conclusion is borne out by a number of observations made while the injury progressed in extent and severity. Of course, it is not definitely known whether the injury observed in this instance came about because of a greatly increased transpiration rate immediately following the application of May 31 or June 25, or both, as is suggested by the work of Duggar and Bonns (9) and Martin (16), or whether the effect was due to a cumulative action over a period of two or three weeks following the spray application. Many other species of plants in similar situations were suffering severely from a lack of water. The symptoms observed were those usually accompanying injury and death due to drouth. Most of the ginseng plantings in wood lots showed severe injury by the end of July, while those depending on lath shelters for shade were not affected and remained green until the usual time in the fall. In the wood lot plantings injury was first noticed in definitely delimited areas and all of the plants in these areas showed similar symptoms of injury within a very short time. These areas were always found to be low in soil moisture, either because of some peculiarity of surface conformation or near-by tree groupings or because they were places subjected to an amount of sunlight far above the average for the planting, as a whole. The subsequent increase in size and number of these areas of injury was definitely connected with a progressive decrease in the soil-moisture content. After 75 per cent of the plants in each of the two wood lots in question had succumbed there were still many small, isolated areas scattered about in which all of the plants were in good condition, even though they had received exactly the same spray and cultivation treatment as the rest of the planting. These areas, usually surrounded by dead or dying plants, were in favorable situations from the standpoint of soil moisture, such as at the foot of slopes or in slight depressions. Finally, what is perhaps most important, all of those beds that had not been sprayed with Bordeaux mixture, such as check plots, dusted plots, and those sprayed with compounds which left little or no visible residue on the leaves, remained in good condition until

the end of the season. These uninjured beds were surrounded on all sides by sprayed plots in which 100 per cent of the plants were dried up.

Thus, the situation may be summarized somewhat as follows: The moisture content of the soil in the wood lots became low in June and at certain times during this month and in July approached the wilting-point value. This, combined with the high evaporating power of the air, frequently resulted in a severe drying of the leaf tissues. Incipient and transient wilting began to occur, but those plants which had not been sprayed were always able to recover before permanent wilting was followed by death. However, the presence of the Bordeaux film on the leaves of the sprayed plants increased the water loss sufficiently at some time during the wilting process to exceed the critical point of desiccation and to render subsequent recovery impossible. Consequently death and drying-out of the tissue resulted. Those plants growing in dry soil areas or standing in greater than the average amount of sunlight were the first to succumb and were followed by others as the conditions affecting desiccation of the tissues became more severe.

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ANTHRACNOSE OF STRAWBERRY CAUSED BY COLLETOTRICHUM FRAGARIAE, N. SP.

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INTRODUCTION

During the years 1926–1929 observations were made upon an anthracnose disease attacking strawberry runners. Although a survey made in 1926 of the fields in the central Florida strawberry-growing area showed that the disease was not widely distributed, surveys made during subsequent years showed a slight increase in the amount of the disease each year. In the year 1930 anthracnose was fairly well scattered throughout the central Florida area. In certain fields anthracnose has been present each year in such abundance that plant propagation has been a failure. The disease attacks mainly the runners and girdles them, thus cutting off the food supply to the young plants before they have rooted and become self-supporting. This reduction in the number of plants propagated is a serious loss to the Florida grower because he depends almost entirely upon the plants he can grow during spring and summer to supply him with plants for fall setting. Northern-grown plants cannot be shipped in satisfactorily at that time.

Briefly, the system of strawberry propagation practiced in Florida is as follows: Each year during February and March strawberry plants are secured from northern nurseries and set out in beds. By June these beds will be covered with runner plants which are removed and set out in other beds. By September these last beds will be covered with runner plants which are removed and set out in the fields where they grow and produce fruit from the last part of November until May, at which time they are plowed under. For every thousand plants which the grower secures from the North in February he raises 20 to 50 thousand plants for fall setting.

Outside of Florida anthracnose apparently has not been observed in any State of the United States. At any rate, reference to this disease has not been found in the literature. Halsted,[1] p. 327–328, in listing some strawberry diseases, mentioned an "anthracnose" caused by *Gloeosporium fragariae* (Lib.) Mont., but Wolf[2] gives this as a synonym of *Diplocarpon earliana* (Ell. & Ev.) Wolf, the organism causing leaf scorch of strawberry.

[1] Halsted, B. D. Report of the Botanist N. J. Agr. Exp. Sta. Rpt. 1893: 289–436.

[2] Wolf, F. A. Strawberry leaf scorch. Jour. Elisha Mitchell Sci. Soc. 39: 141–164. 1924.

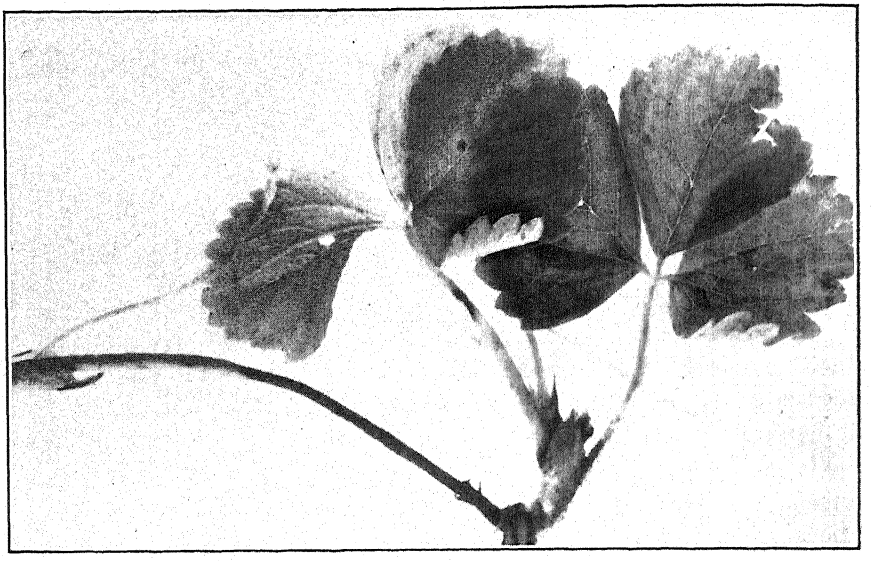


FIG. 1. Lesions on strawberry runner and petiole produced by *Colletotrichum fragariae* under field conditions.

THE DISEASE

Symptoms. Under field conditions anthracnose most commonly affects the runners, the petioles being affected occasionally. The young lesions are dark brown, oval, and 1 to 2 mm. long. They increase in size longitudinally for several centimeters and laterally until the runners are girdled (Fig. 1). Under favorable environmental conditions, high temperature and abundant moisture, these lesions may extend throughout the length of the runner. Ordinarily, however, the average lesion extends for 1 to 2 cm. along the runner and girdles it. These lesions are typically anthracnose-like, sunken, dark brown to black, and sharply demarcated from the surrounding healthy tissue. The cortex in the older portion of the lesion becomes shrivelled, and over its surface are scattered groups of setae which can readily be seen in profile with the aid of a strong hand lens. Microscopic mounts of strips of epidermis peeled from these lesions show a scattering of numerous acervuli, either bordered by or interspersed with dark brown setae (Fig. 2, A). Conidia are produced in abundance in the acervuli.

Seasonal Development. Anthracnose is most abundant in central Florida during the rainy season, June to September. It has been found as early as May, but it is not seen during the late fall and winter months because, at that time, the strawberry plants are kept free of all runners.

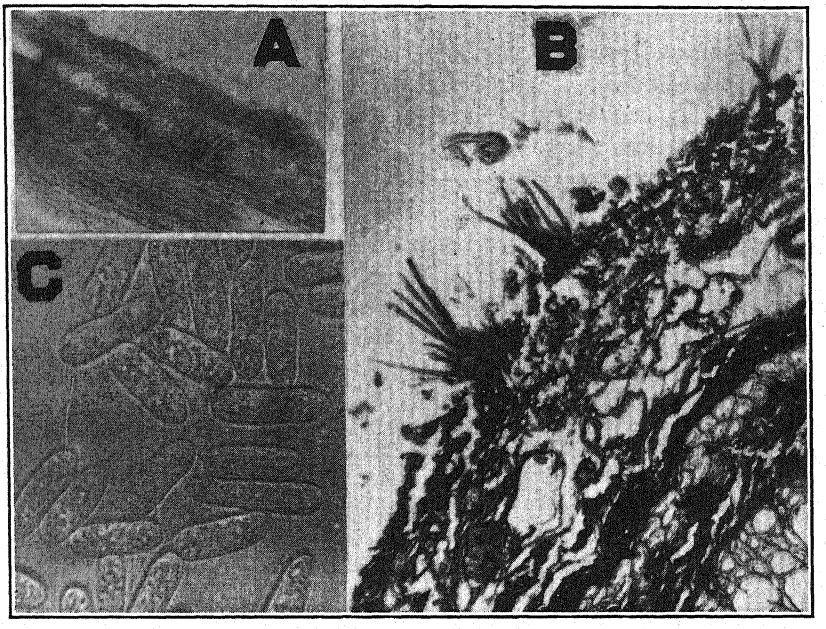


FIG. 2. Photomicrographs of *Colletotrichum fragariae:* A. Surface view of runner lesion, showing setae in acervuli. ×100. B. Cross-section of strawberry runner, showing disorganization of cortical cells caused by the fungus. ×200. C. Conidia. ×1,000.

In individual fields anthracnose shows up to a greater extent in the lower areas where water accumulates during heavy rains. Thus, surface water is one agency for the distribution of spores.

CAUSAL ORGANISM

Isolation. Suspensions of conidia from diseased runners were used for the first inoculation experiments from which typical symptoms of the disease developed upon healthy runners. Later, cultures of the organism were obtained upon lima-bean agar from single spores. In culture, conidia were produced abundantly in 6 to 8 days, and when used to inoculate healthy runners they produced a disease which compared exactly with that found under field conditions. The organism was reisolated from the artificially inoculated plants, grown in culture, and it again produced the disease when inoculated into healthy runners.

Cultural Characteristics. Pure cultures of the causal organism were grown on corn-meal, oatmeal, and lima-bean agars, each containing 1 per cent glucose. Inoculated agar plates showed growth within 12 hours, and the average rate of growth was 0.40 to 0.46 mm. per hour at atmospheric temperatures ranging between 75° and 90° F. Vegetative growth and production of conidia were scant upon corn-meal agar but more profuse upon oatmeal and lima-bean agar. The growth of the organism on all of these

media was black and zonate, with short, cottony, aerial hyphae, and the conidia appeared as pink masses. Cultures grown upon artificial media for a period of 6 months were found to have lost their pathogenicity.

Cultures on autoclaved string beans and strawberry runners gave an abundant growth of fairly long, cottony hyphae, with some production of conidia.

Taxonomy. Because of the symptoms of this disease and because it is caused by a species of Colletotrichum, the common name "anthracnose" is suggested, a term which the writer has applied to the trouble since its discovery and which will be retained since the "anthracnose" mentioned by Halsted is now called "leaf scorch."

The species of Colletotrichum causing anthracnose of strawberry runners has been compared with other described species of that genus and was found to differ from all of them. Host plants of species of Colletotrichum most like the one causing the strawberry anthracnose were inoculated with pathogenic cultures of the latter organism, but no infections resulted. The plants tested were alfalfa, hollyhock, red clover, snapdragon, spinach, and string bean.

A survey was made for wild hosts of the organism which causes strawberry anthracnose, but none has been found. Inoculations into the following plants failed to produce the disease: *Agrimonia* sp., blackberry, dewberry, *Gerardia* sp., *Ludwigia virgata* Michx.

Successful inoculations were made into runners of *Duchesnea indica* (Andr.) Focke.

No ascigerous stage of the fungus has been found either on host tissue or in culture. The strawberry anthracnose organism is therefore presented as a new species and the binomial *Colletotrichum fragariae* assigned to it.

Colletotrichum fragariae, n. sp.

Lesions brown to black, sunken, one to several centimeters long, girdling runner. Acervuli erumpent, scattered, mostly lenticular, 70–140 μ long by 30–60 μ wide (av. 110 x 40 μ). (Fig. 2, A, B, and 3, A.) Setae few to abundant, occurring singly or in groups, somewhat sinuous, 1 to 2 septate, 97–142 μ long by 3.8–5.4 μ wide (av. 115 x 4.3μ), dark brown and subbulbous at the base becoming lighter toward the apex, sometimes with a small, slightly constricted apical cell. (Fig. 3, B.) Conidia abundant, 14–21 μ long by 3.9–6.3 μ wide (av. 16.4 x 4.8 μ), spindle to boat-shaped with rounded ends, granular, 1 to 2-guttulate, pink in mass. (Fig. 2, C.) Conidiophores 5–10 μ long by 3–5 μ wide (av. 7 x 3.5 μ), hyaline, nonseptate, ovoid.

INFECTION EXPERIMENTS

Inoculation experiments were performed to determine the environmental conditions under which infection takes place and to determine whether leaves and petioles would become infected.

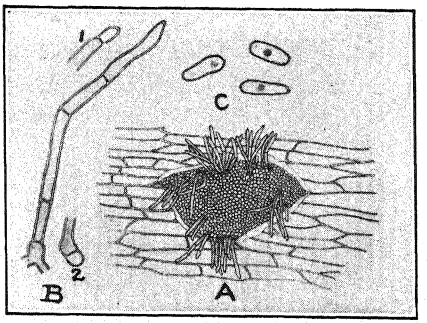


FIG. 3. Camera-lucida drawings of *Colletotrichum fragariae*, showing: A. Acervulus with arrangement of setae; B. Seta, with constricted apical cell (1) and subbulbous base (2); C. Conidia.

During September and October, 1927, runners on potted strawberry plants were inoculated with spore suspensions of the anthracnose organism. On one set of runners a fine needle was used to prick through the inoculum into the plant tissue; on the other set there was no mechanical injury made. Subsequent to inoculation, half of the plants of each set were placed in a moist chamber and half in partial shade in outside atmosphere. The results of these inoculations are summarized in table 1.

TABLE 1.—*Incubation period of Colletotrichum fragariae on strawberry runners, as affected by method of inoculation and treatment of plants subsequent to inoculation*

Method of inoculation	Treatment subsequent to inoculation	Incubation period—days
Inoculum pricked into epidermis	Placed in moist chamber	2– 3
	Placed in outside atmosphere and partial shade	5– 7
Inoculum placed on uninjured epidermis	Placed in moist chamber	6– 8
	Placed in outside atmosphere and partial shade	12–14

Another set of inoculation experiments was performed to determine the susceptibility of runners of different ages and portions of runners to anthracnose. From these experiments it was learned that the younger, more succulent runners or parts of runners were more readily invaded by the fungus than were the older ones, the tip of the runner being most susceptible. These results confirm observations which were made of the disease under field conditions.

Leaves and petioles of healthy strawberry plants also were inoculated. Some of the inoculations were made by placing a drop of spore suspen-

sion on the plant part and pricking through it into the plant tissues, others by merely placing the drop on the plant part. Runners were inoculated at the same time to serve as checks upon the pathogenicity of the culture used. Disease symptoms appeared to a limited extent on the leaves and petioles which had been injured, but no signs of infection developed on those which were inoculated without wounding. Both sets of runners which were inoculated under similar conditions developed symptoms of the disease. The causal organism was reisolated from the leaves and petioles showing the disease symptoms.

CONTROL

As has been stated previously, anthracnose girdles the runners and causes the death of the young plants which have not yet put out roots and become self-supporting. It thus causes considerable loss during the period of plant propagation—July through September. Applications of Bordeaux mixture 4–4–50, at 10-day intervals during the summer months have been found to check the progress of the disease but not to give complete control. The frequent rains at that time make control difficult.

Proper drainage of the strawberry nursery beds will help to check the spread of anthracnose.

Crop rotation may be an aid in combating this disease.

SUMMARY

(1) Strawberry anthracnose caused by a species of Colletotrichum has been found under field conditions mainly on the runners of this plant and only occasionally on petioles. It has been produced artificially upon petioles and leaves by inoculating through wounds.

(2) The disease has been observed only in the strawberry-growing area of central Florida.

(3) The disease is characterized by dark brown to black lesions on the runners. It causes considerable damage by girdling them and preventing the development of new plants.

(4) The causal organism has been isolated and its pathogenicity has been proved by Koch's postulates.

(5) The causal fungus is a species of Colletotrichum which is different from any known described species. A description of it is given under the proposed binomial **Colletotrichum fragariae**, n. sp.

(6) Bordeaux mixture 4–4–50, applied at 10-day intervals during the summer, checks the spread of the disease but will not completely control it, because of the abundant rainfall at that time.

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INFLUENCE OF MOSAIC INFECTION ON TOMATO YIELDS

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INTRODUCTION

The literature relating to the mosaic disease of the tomato is replete with statements of observations on the reduction in yield being associated with time of infection. Gardner and Kendrick (1) state: "It is a matter of common observation that many plants infected early in the season may show very extreme effects of mosaic and bear no marketable fruit whatever. Others may bear a greatly reduced yield, while, in the case of plants infected late in the season, the effect on the yield is not very noticeable." In a later paper (2) these investigators again call attention to the fact that early infections cause the greatest loss. McKay (5) observed in Oregon, under both field and greenhouse conditions, that yields from mosaic-infected plants varied greatly, some yielding about a third of an average crop while others produced apparently an average yield. However, the literature reveals very little exact data upon the correlation of these losses with the time of infection. Norton (7) showed by measurements in the greenhouse that, although 33 per cent more fruit set on the plants longest remaining healthy, they produced very little more weight of fruit than plants infected earlier. McCubbin (4), under field conditions in Ontario in 1915, showed that 59 healthy plants gave 36.8 per cent more fruit and 40.5 per cent more weight of fruit than 59 mosaic plants. However, he does not state when his mosaic plants were infected. Unpublished observations made by J. B. S. Norton and R. A. Jehle during several years in Maryland tomato fields showed lower yields from the earlier infected plants. The investigations reported in this paper were undertaken with the object of obtaining more exact data on the effect of early and late infections of the common type of tomato mosaic on the yield of tomatoes under Maryland conditions. Two types of experiments are reported. One in 1927 showed the decreased yields based on the time of appearance of mosaic symptoms, and the other, in 1930, showed the decreased yields based on date of inoculation at definite growth stages.

METHODS AND RESULTS

1927 experiment: On July 1 a careful examination in a plot of Greater Baltimore tomato plants 6 to 8 inches tall, planted June 10, was made for individuals showing mosaic symptoms. The location of infected plants was

[1] The writers wish to acknowledge their indebtedness to Dr. J. B. S. Norton, under whose direction this work was done, for his valuable suggestions and criticisms.

recorded on a chart. Thereafter, 6 examinations at intervals of 1 week were made and, from the data obtained, 10 plants were selected from each weekly survey for yield studies. Harvests of ripe fruit were made at intervals of 1 week from August 22 to September 22, inclusive. Total yields for each of 6 series, based on the time of appearance of mosaic symptoms, were obtained. The average yield per plant for each series is presented in table 1. Under existing field conditions it was not possible to find by the first harvest 10 mosaic-free plants. Therefore, the yield of those plants which showed infection on August 12 was used as the basis for loss calculations. The 1927 growing season was approximately an average one.

TABLE 1.—*Summary of yield records of infected tomato plants comprising the 1927 experiment*

Time of appearance of symptoms	Number of plants	Average yield per plant in grams	Percentage loss due to mosaic
July 8	4	1,993	56.9
" 15	10	2,854	38.2
" 22	10	3,488	24.5
" 29	10	4,122	10.8
Aug. 5	10	4,532	1.9
" 12	10	4,621	.....

1930 experiment: Two series of 3 plots, each, were laid off side by side on a comparatively level piece of sandy loam soil. In each series 2 plots were planted to the Marglobe variety and 1 plot to the variety Greater Baltimore. Half of the plots of each variety was used for inoculations and the other half for controls. Similar fertilizer treatments and cultural practices were employed throughout.

Plants were obtained from beds apparently free from mosaic and were set on June 21 four feet apart in rows 4 feet apart. These plants were about a week older than the usual age for transplanting. The late planting was due to the fact that the soil was not previously available. During the transplanting process the hands of the workers were frequently washed with soap and water, and only strong, vigorous, healthy plants were set out.

All experimental inoculations were made by a slight modification of the needle-prick method devised by Holmes (3). Four inoculations were made during the growing season: the first, at transplanting time; the second, when the first flower cluster had formed; the third, when the first fruits

set were ½ to 1 inch in diameter; and the fourth, when the first fruits were ripe.

All precautions were taken to prevent the spread of mosaic by mechanical means. The field was inspected at weekly intervals for the appearance of cases of accidental infection and for the presence of aphids. Aphids were scarce throughout the season except for a small number being present just after the plants were set out. The accidental spread of mosaic was unusually slow and, at the end of the growing season, 51 plants in the control plots were still healthy. These accidental cases of infection are not included in the data.

Harvesting began on September 3 and continued until October 3. The yield records are summarized in table 2.

Drought conditions prevailed throughout the season and, for the months of June, July, August, and September, a deficiency of 11.01 inches of rainfall occurred, there being during this period only 29.47 per cent of the average rainfall.

TABLE 2.—*Summary of yield records of healthy and inoculated tomato plants comprising the 1930 experiment*

Date of inoculation	Number of plants used	Average number of fruits set per plant	Average yield per plant in grams	Percentage loss due to mosaic
First (6–26–30)	39	21	685	54.4
Second (7–11–30)	39	24	836	44.4
Third (8–14–30)	27	32	1,122	25.3
Fourth (9– 3–30)	13	32	1,335	11.2
Control plants	51	36	1,503	.....

In 1929 a preliminary test was made on the effect of mosaic on the quality of the canned fruit. Six cans each of healthy and mosaic fruit were prepared and then scored by an expert scorer, using the score sheet for canned tomatoes, tentative draft of January, 1928, of the National Canners Association. The differences between the healthy and mosaic cans were negligible.

DISCUSSION

Although these experiments were conducted under different climatic conditions, practically identical results were obtained. In 1927 the greatest loss in yield occurred with plants showing symptoms by July 8, while in 1930 the plants inoculated 5 days after transplanting produced a de-

cidedly lower yield than those inoculated later. In both experiments later infections produced similar results—the later the time of infection the less the reduction in yield. The results obtained show conclusively that the decrease in yield varies in direct relation to the earliness of infection. McMurtrey (6) and Valleau and Johnson (8), working with tobacco, obtained the same relation.

Although the results of the later infections show the same relation, they differ quantitatively in the 2 years, and these differences seem to bear a definite relation to cultural practices and climatic conditions. The plants used in 1927 were transplanted about 2 weeks earlier and were somewhat younger and smaller than those used in 1930. Hence, during the former season with approximately average rainfall the plants grew faster, matured fruit earlier, and produced greater yields than the 1930 plants, grown under drought conditions. These differences indicate that late infection of plants of retarded growth causes more damage than late infection of plants of average growth. For example, the figures in table 1 show only a 10.8 per cent loss when symptoms appeared on July 29, infection approximately 12 days earlier, while the figures in table 2 show that infection on July 11 resulted in a 44.4 per cent loss.

The figures presented in table 2, for the average number of fruits set per plant, show that the first two infections resulted in less fruit being set than the last two infections, while the healthy plants set the most fruit.

SUMMARY

Both experiments show that the early infections cause the greatest reduction in yield and that a direct relationship exists between the time of infection and the amount of reduction in yield. The figures presented in the tables show that the earliest infection in both experiments caused a reduction of over 50 per cent in yield, while the last infection caused a reduction in yield of 1.9 per cent in 1927 and 11.2 per cent in 1930.

Late infections in both experiments produced similar results, but the results differed quantitatively in the 2 years. A probable explanation is given for these differences.

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HETEROTHALLISM IN CORN RUST AND EFFECT OF FILTERING THE PYCNIAL EXUDATE[1]

GEORGE B. CUMMINS[2]

Since the presence of heterothallism in the Uredinales was discovered by Craigie (3) the question of just what takes place to cause the formation of aecia has remained unanswered. Craigie (4) subjected the pycnial exudate to a temperature of 70° C. and found that no stimulatory action was retained, the temperature supposedly being sufficient to kill the pycniospores. From this he concluded that the living pycniospores were essential. Arthur (2, pp. 242–243), in discussing the question, raised the valid objection that these results were inconclusive, as a temperature of 70° c. is considerably above that necessary to inactivate most enzymes. Since enzymes or similar substances are known to be present in the pycnial exudate, he suggested that these may furnish the necessary stimulation for aecia formation.

By the use of Craigie's methods, it has been found that corn rust, *Puccinia sorghi* Schw., which has as its alternate host, oxalis (*Xanthoxalis stricta* (L.) Small), is apparently heterothallic. In unpublished work Mains had previously reached the same conclusion. Oxalis plants were subjected to infection from suspended hibernated telial material and were then moved to cloth-covered cages to reduce the chance of contamination by insects after pycnial pustules appeared. Sterile needles were used to transfer exudate from one pustule to another. Of the 52 pustules treated by transfer of exudate, 39, or 75 per cent, formed aecia and 13 did not. Of the 42 pustules left untouched, 6, or 14.3 per cent, formed aecia and 36 did not. Thus, mixture of the pycnial exudates definitely stimulated aecia formation.

In order to determine whether or not the formation of aecia might be due to the presence of some enzyme in the pycnial exudate, the latter was filtered to remove the pycniospores. In preparation for this, two groups of oxalis plants were inoculated, one heavily, to serve as a source of exudate, and one very lightly. The lightly infected plants were immediately placed in cloth cages and kept separate from the heavily infected plants. When pycnial exudate became abundant, it was removed from the heavily infected plants by scraping the pustules with a flattened needle or a small scalpel. The exudate thus collected was mixed with a few cubic centi-

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station, La Fayette, Indiana.

[2] The writer makes grateful acknowledgement to Dr. E. B. Mains for suggesting the problem and to Dr. J. C. Arthur for helpful criticism of the manuscript.

meters of distilled water to form a relatively concentrated mixture. Part of this was filtered through a small Berkefeld filter and the filtrate collected in a small glass vial.

Because of the small quantity of the mixture a modification of the usual method of filtering was employed. The method and the apparatus are essentially those of Martin, as described and illustrated by d'Herelle (6, pp. 22–23). A 2-inch Berkefeld filter of small diameter was suspended in a 250 cc. evacuation flask and the metal shaft was inserted into a small rubber stopper, which, in turn, could be inserted into a glass tube of suitable diameter. This tube was run through a rubber stopper of the size required by the flask. A small vial was tied over the lower end of the filter and the mixture was poured into the filter through the glass tube. By applying suction a small amount of the mixture could readily be filtered. In order to insure the efficiency of the filter, it was tested with a bacterial suspension. To accomplish this the entire apparatus was sterilized in the autoclave; the bacterial suspension was then filtered and the filtrate plated under sterile conditions. The fact that no bacterial colonies developed on the agar plates was considered sufficient proof that the apparatus was filtering properly. The filtered pycnial exudate was carefully examined with a microscope and no pycniospores were found.

On the lightly infected group of oxalis plants certain isolated pustules, presumably of monosporidial origin, were treated with the filtered exudate and others with the nonfiltered exudate. The exudate was applied with needles that were sterilized between each operation. The plants were then kept in cages until aecial counts were possible. Of the 10 pustules treated with the nonfiltered exudate all produced aecia in the usual time, as would be expected, since the mixture contained exudate from many pycnia. Of the 28 pustules treated with the filtered exudate none showed aecia in the usual 10-day period but 8 developed scattered aecia when held for an additional 1 to 3 weeks, though the aecia which actually opened and discharged spores usually were few and scattered on any one area.

Why 8 of the pustules treated with the filtered exudate produced aecia is unknown, but in all such experiments on the rusts a few scattered aecia have appeared where they were not expected, especially if held beyond the time required for normal aecia formation. Craigie (5) found that delayed development of aecia in nontreated pycnial pustules in *Puccinia graminis* Pers. was not uncommon, and Allen (1) reports irregular appearance of aecia in otherwise sterile pustules.

The results here reported indicate that *Puccinia sorghi* is heterothallic and that the formation of aecia cannot be explained on the basis of an enzymatic stimulation, as the pycnial exudate, which was freed from

pycniospores without inactivating its enzymes, lost its stimulatory action, and therefore substantiate Craigie's conclusion that the presence of living pycniospores is essential.

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NEW OR UNUSUAL SYMPTOMS OF VIRUS DISEASES OF RASPBERRIES

GEORGE L. ZUNDEL

While working on the control of virus diseases of raspberries in Pennsylvania, some unusual reactions have been encountered, which, it seems to the writer, may be of more than passing interest.

In the spring of 1929, the raspberry patch of A. S. Seip, Wernersville, Pa., showed in an old planting of Cumberland raspberries an unusual type of mosaic to which the writer has since applied the term "fern-leaf" mosaic. The leaves were dwarfed and the tissue between the veins was wrinkled and unusually dark green. The serrations of the leaflets were accentuated, giving them a fern-leaf appearance. All parts of the infected plants, including the canes, were very brittle, and the plant appeared much dwarfed. The general appearance of such a plant is shown in figure 1, A.

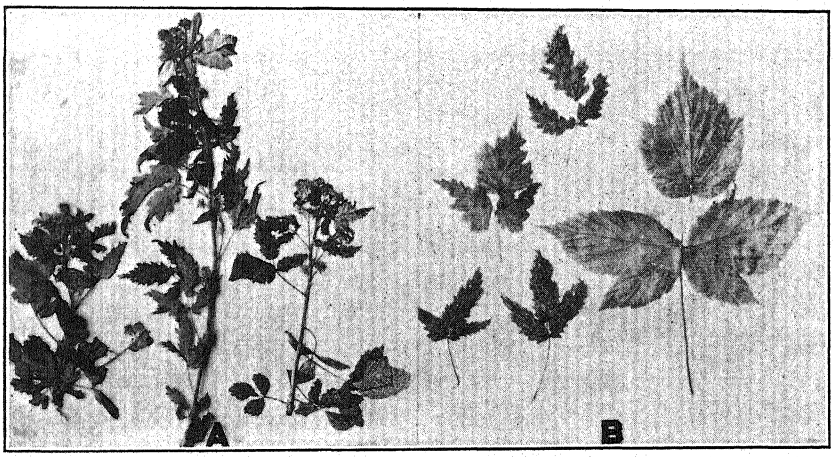


FIG. 1. "Fern-leaf" of raspberry. A. Diseased twigs. B. Diseased leaves compared with a healthy leaf.

In figure 1, B, is shown the relative size of diseased and healthy leaves. The fruit also was dwarfed, dry, crumbly, and tasteless.

Mr. Seip is the only farmer in his locality who grows melons and cucumbers. For several years past his cucurbit patches have been adjacent to the infected raspberry patch. It is therefore suspected that the fern-leaf symptom is the result of infection of the raspberries by the virus

of cucurbit mosaic. This assumption is based upon the fact that Mogendorff[1] has recently proved that fern-leaf mosaic of tomatoes is caused by the transfer of cucurbit mosaic to tomato plants. The similarity of the symptoms on the raspberry and on the tomato will be seen by comparing figure 1, A, with figure 2, A, which is a photograph of fern-leaf mosaic of



FIG. 2. "Witches'-broom" of respberry. A. General appearance of diseased plant. B. Showing detail of "witches'-broom" together with abnormal amount of foliage.

tomatoes collected in the greenhouse of the Pennsylvania State College from a plant adjacent to the cucumber bed.

Another interesting case was found in the Cumberland raspberry patch of John G. Dietz, Ridgway, Pa., July 24, 1930. Attention was attracted to a single plant having an unusual amount of foliage. An examination showed that most of the side branches of the canes had been replaced by a large number of "spindle sprouts" forming a witches'-broom type of growth. Many of the spindle sprouts were 12 inches long. The plant was 3 feet tall and had produced an unusually large crop of dry, bitter berries which had entirely lost all normal flavor. The canes had the characteristic blue color, and many of the leaves showed the usual downward curling of the tips also characteristic of streak. While witches'-brooms have never been described in connection with streak, it seems probable from the color of the canes and the leaf character that this is merely an unusual manifestation of the streak virus. Mottling of the leaves was not very evident.

[1] Mogendorff, N. "Fern-leaf" of tomato. Phytopath. 20: 25–46. 1930.

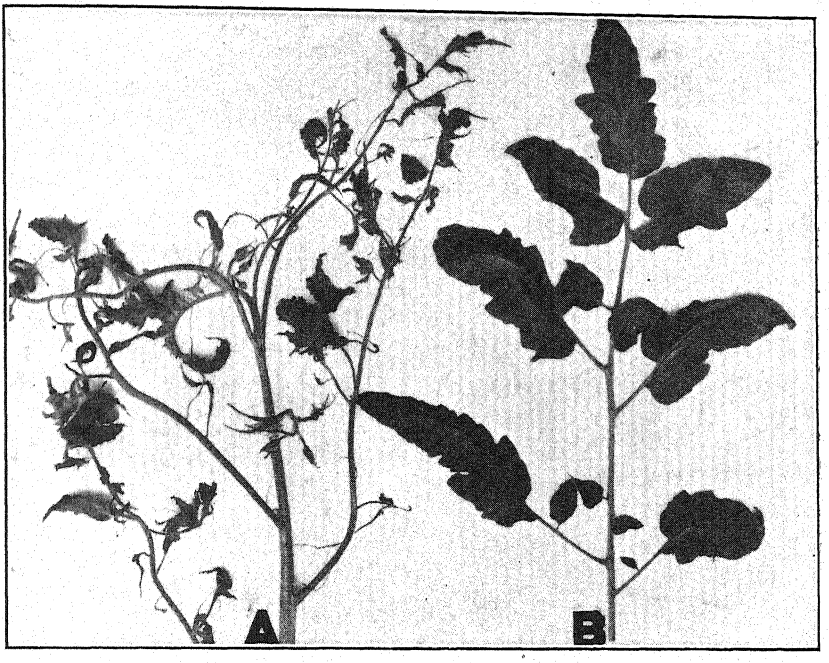
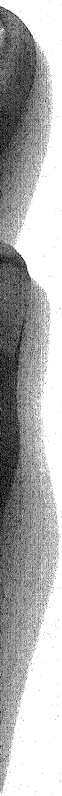


FIG. 3. "Fern-leaf" of tomato. A Diseased branch. B. Healthy leaf.

Figure 3, A, shows the general appearance of the plant; figure 3, B, shows the witches'-broom growth, together with the unusually abundant foliage.

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PHYTOPATHOLOGICAL NOTES

Tylenchus dipsaci on Hypochaeris radicata in Hawaii.—The bulb- and stem-infesting nematode *Tylenchus dipsaci* was reported by Godfrey and McKay[1] on *Hypochaeris radicata* along the Pacific Coast, all the way from Washington into California. More recently (1926 and 1928) the writer has found it on this same weed on the lawns at the University of California. In the fall of 1930 this plant occurred in great abundance in meadows at Olinda, Maui, on the slopes of Haleakala, at an altitude of 4,000 feet. A casual observation disclosed the fact that more than 50 per cent of the plants examined were heavily infested with the stem nematode. (Identification of the organisms was verified by examinations in the laboratory.) Typical swellings were found in leaf blades and midribs and in flower

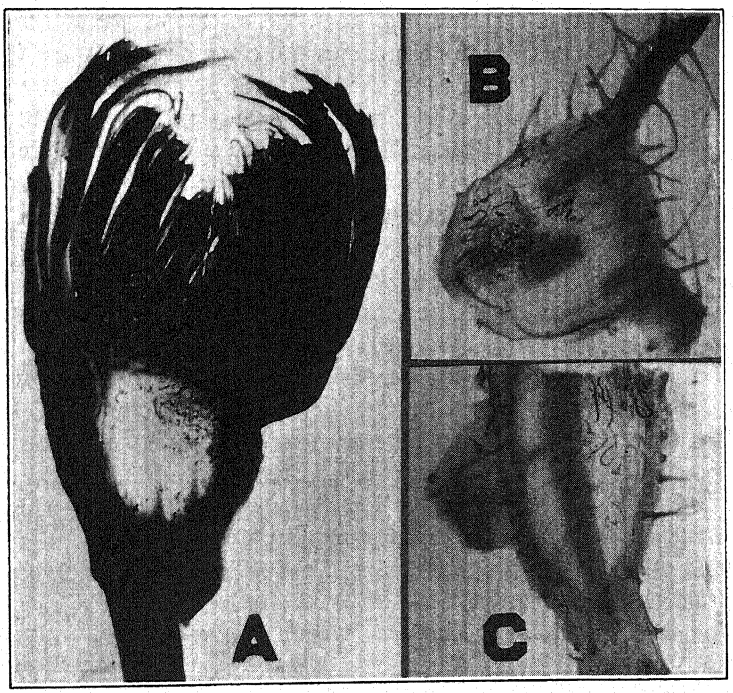


FIG. 1. *Tylenchus dipsaci* in *Hypochaeris radicata.* A. Longitudinal section through a flower head, showing heavy infestation with nematodes within and beneath the receptacle. B. Section through a swelling on the side of a leaf midrib, showing a "pocket" of nematodes within the leaf tissues. C. Section showing a similar pocket in the leaf mesophyll. All materials killed in Flemming's solution and cleared in clove oil, to show nematodes in their natural position.

1 Godfrey, G. H., and M. B. McKay. The stem nematode, *Tylenchus dipsaci,* on wild hosts in the Northwest. U. S. Dept. Agr. Bul. 1229. 1924.

peduncles. Many infested flower heads were found, as with those reported by Godfrey,[2] in this plant and in true dandelion, *Taraxacum officinale*. There is little doubt that extensive distribution in the infested region has occurred by means of seed transmission. Whether or not original infestation came to the Islands by this means is an interesting conjecture. The accompanying photographs of plant materials killed with Flemming's solution and cleared in clove oil show to good advantage the nature of the nematode infestation.—G. H. GODFREY, Experiment Station, Association of Hawaiian Pineapple Canners, Honolulu, T. H.

PROFESSOR McGINTY *RE* PHARMACIEN FRECHOU[1]

Among the papers published in PHYTOPATHOLOGY occasionally there is one which strengthens the conviction that, while some of the older generation of mycologists may have been shown the error of their ways through the pointed comments of my late friend C. G. Lloyd, our more recent workers, alas, require a like discipline. For example, in the April number there is a brief note by a William H. Weston, Jr., written in a style tinged here and there with unseemly frivolity and showing greater interest in the merely human than in the scientific aspects of investigation, which begins with the sentence "It was in 1878 and 1879 that *Sclerospora graminicola* (Sacc.) Schroet. was first reported from Italy, from France, and from Germany." Now I do not know much about this writer, save that he apparently is afflicted with the urge to add his name to species of Sclerospora, but I notice that he is quite ready in this note to criticize the work of others and so he should welcome my calling attention to the following facts in connection with his opening sentence. It was in 1876 that P. A. Saccardo in his "Fungi Veneti Novi Vel Critici," Series 5, in the Nuovo Giornale Botanico Italiano 8, p. 172, described *Protomyces graminicola* which had been collected at Selva in September, 1875, on *Setaria verticillata* and listed as No. 496 in his "Mycotheca Veneta"; while, in 1875, J. Urban in Lichterfelde, near Berlin, had collected similar material on *S. viridis* which he sent to P. Magnus with a letter of description on the basis of which Magnus described *Ustilago* (?) *Urbani* in 1879; and in August, 1877, at Frederickshain, near Berlin, E. Ule collected the same fungus which was issued under *U. Urbani* of Magnus as No. 2498 of Rabenhorst's "Fungi Europeae."

[2] Godfrey, G. H. Dissemination of the stem and bulb infesting nematode, *Tylenchus dipsaci*, in the seeds of certain composites. Jour. Agr. Res. 28: 473–478. 1924.

[1] Published at the expense of William H. Weston, Jr., out of the order determined by the date of receipt of the manuscript.

Moreover, even though Weston, in 1921, had indeed called attention in his paper on "Production and Dispersal of Conidia etc." to Prillieux's report of germination, he seemed to have forgotten his own reference to it by 1928, for in the Journal of Agricultural Research, June, 1928, in connection with the downy mildew on Everglade millet in Florida, he says (p. 954) of the oospores of *Sclerospora graminicola*, "The part these spores play in the life history of the fungus has not been worked out in all its details and the actual method of germination has not been reported or investigated." It would be better for mycology if writers such as the one in question paid more attention to strict accuracy in the details of their publications rather than to imaginative speculations as to the activities of pharmacists long since dead.—PROFESSOR ALOYSIUS T. MCGINTY.

The renewal of Professor McGinty's activities should be hailed with interest and enthusiasm by all mycologists and plant pathologists. In this instance his criticism is entirely just.—WILLIAM H. WESTON, JR., Laboratories of Cryptogamic Botany, Harvard University.

Occurrence of Cadophora fastigiata in Canada.—In their work on blue stain in pine and spruce in Sweden, Lagerberg and Melin[1] describe for the first time the fungus *Cadophora fastigiata* L. et M., nov. gen. et sp., and report its widespread occurrence in that country as a causal agent of blue stain in softwoods. Otto Kress *et al.*,[2] in their study of decay in pulp, had previously described and figured an unnamed fungus which they had frequently found causing grey spotting of ground wood and sulphite pulps in the United States. Lagerberg and Melin consider this fungus to be identical with their *C. fastigiata*. The fungus is represented, therefore, in both Europe and America.

Cadophora fastigiata has recently been isolated by the writer from stained sapwood obtained in Canada from the following species: White spruce, *Picea canadensis* (Mill.) B. S. P.; white pine, *Pinus strobus* Linn.; jack pine, *P. Banksiana* Lam.; and Douglas fir, *Pseudotsuga taxifolia* (Lam.) Britton.

When grown in pure culture on sapwood blocks of white pine, *Pinus strobus*, and red pine, *P. resinosa* Aiton, *Cadophora fastigiata* produced an intense grey-green stain. It also stained to some extent culture blocks of black spruce, *Picea mariana* (Mill.) B. S. P., sapwood.

It is very probable that fungus is responsible for a considerable amount of the sapwood stain found in Canadian softwood lumber.—E. A. ATWELL, Forest Products Laboratories of Canada, Ottawa, Canada.

[1] Lagerberg, T., G. Lundberg, and E. Melin. Biological and practical researches into blueing in pine and spruce. Svenska Skogsvårdsför. Tidskr. 25: 145–272. 1927.

[2] Kress, Otto, *et al.* Control of decay in pulp and pulpwood. U. S. Dept. Agr. Dept. Bul. 1298. 1925.



BOOK REVIEWS

Maximov, N. A. A Textbook of Plant Physiology. Edited by A. E. Murneek and R. B. Harvey. vii–xvi + 381 pp., 152 figs. Edition I. McGraw-Hill Book Company, Inc., New York and London. 1930.

This English translation of the first edition of Dr. N. A. Maximov's "Textbook of Plant Physiology" constitutes an interesting addition to our relatively few texts on plant physiology. Dr. Maximov is a professor in the Pedagogical Institute of Leningrad and Director of Plant Physiology of the Bureau of Applied Botany, U. S. S. R. His world-wide recognition as an authority on the ecological relations of plants, especially in the field of water relations and drought resistance, makes his book of more than ordinary interest.

Following an introduction that points out the close interrelationships of plant physiology with other sciences, both fundamental and applied, there are four major divisions. The first, entitled "Absorption of Matter and Energy," deals with the plant's synthesis of organic compounds from inorganic materials. The sources and utilization of carbon and nitrogen and of mineral elements are discussed in the three chapters of this section. The second major division, "Water Relations of the Plant," is of special interest, embodying the results of the author's distinguished contributions to knowledge in this field. After a chapter dealing with the concept of the cell as a colloidal osmotic system, there are three chapters dealing with the absorption, loss, and translocation of water in the plant. The third division, "Utilization of Reserve Products and Liberation of Energy," begins with a chapter dealing with the storage of substances in seeds and other organs, their mobilization through the agency of enzymes, their utilization in the production of new living matter, and their deposition as food reserves in the seed at the end of the cycle. The second chapter in this section deals with respiration. The fourth and final major division, "Growth, Movement, and Reproduction," carries the story through the outward manifestations of the absorption, production, and utilization of nutritive substances.

This text has many advantageous features that will recommend it to certain groups. It seems specially adapted to those whose primary interests are in the field of ecology rather than in the physico-chemical aspects of plant physiology. The data and illustrations are frequently drawn from the fields of agriculture and forestry, and the text emphasizes the applications of theory to the applied science. There is a very thorough treatment of the water relations of plants, including an exposition of the fundamental properties of colloids. There are excellent discussions of drought and of

cold resistance and of the practical problems of plant propagation. Phytopathologists will find good discussions of the physiology of parasites and saprophytes.

There seem to be no serious criticisms to be made of the content of the book. Some subjects receive scantier attention than those specially interested in them might wish, such as physiological correlation, photoperiodism from the standpoint of its formative effects on growth, and the significance and nature of the buffer system or acid-regulating mechanism in plants. Those who do not favor the assumption of special hormones to explain the different physiological processes involved in growth and reproduction will criticise the frequent occurrence of this word in the last three chapters of the book. On the whole, there is considerable reserve in referring to the newer contributions, although half a page is devoted to a discussion of the unsubstantiated work of Popoff on growth stimulants.

A few inaccurate statements might be noted here. On page 126 typographical errors are probably responsible for the omission of minus signs in the statements that winter cereals are able to stand 15 to 20° C. and that certain dormant organs, including the needles of conifers, freeze at 15 to 20° C. A misplaced semicolon in the third sentence on page 95 is responsible for an unfortunate ambiguity. In the discussion of amylose (p. 204) the characteristics of the two forms, α and β, have been confused. The relatively insoluble "skeleton" of the starch grain which gives a violet color with iodine is known as α-amylose or amylopectin, while the β-amylose is the more soluble "basic mass" of the grain. On page 345, the use of the words "struggle" and "antagonism" would seem to convey an improper conception of the chemical interrelationships involved in the transition from vegetative to reproductive phases of growth. On page 69 the author doubtless meant to say that the inorganic phosphorus in the plant is largely in the form of phosphates, not free phosphoric acid.

On the whole, the text is well organized and interestingly written, although its usefulness would be increased by a more complex index. For instance, casual inspection has shown that the subjects of aluminum and iron availability, amylose, hydrogen-ion concentration in plants, and at least six references to hormones are not included in the index.

The illustrations are not very good. The half tones are mediocre, and the drawings, often redrawn from other sources, are poorly executed. Also, some of the illustrations are inadequately explained.

From the reviewer's point of view, a fault in the book lies in its failure to include citations to literature. Of the authorities mentioned by name, many are Russian. Complete citations to this literature would add to the value of the book to investigators, but the absence of even a date to

help locate these sources distinctly lessens the usefulness of the book in this respect. At the head of the scanty list of reference books and periodicals, it is stated that those in Russian are omitted because students in elementary plant physiology are not as a rule prepared to read effectively scientific articles in a foreign language. Many will doubt whether the omission of citations to this literature, specifically referred to in the introduction by the editors as a "pedagogical merit," is desirable. It would seem that a student mature enough to be in a university course would not have his attention unduly distracted by references to literature.

While the author shows himself in sympathy with the immediate problems of crop improvement, at the same time he is obviously aware of the value of fundamental discoveries such as those being made in the fields of theoretical physics and chemistry. References to the quantum theory in the discussion of the mechanism of photosynthesis, to the Donnan theory of membrane equilibrium to explain the entrances of substances from the soil into roots, and the application of the facts of colloid chemistry to the problems of the water relations of plants are typical of discussions which indicate his familiarity with the recent concepts of the analytical as well as the descriptive phases of plant physiology. While it may be said that some of the discussions are characterized by overemphasis of the author's own point of view, it should be added that on more than one of the moot questions he has qualified himself to speak with authority.—ANNIE M. HURD-KARRER, Bureau of Plant Industry, Washington, D. C.



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SPORE GERMINATION OF PUCCINIA GLUMARUM WITH NOTES ON RELATED SPECIES[1]

J. M. RAEDER AND W. M. BEVER[2]

INTRODUCTION

According to Humphrey, Hungerford, and Johnson (13), stripe rust, *Puccinia glumarum* (Schm.) Eriks. and Henn., was first described in Europe by Schmidt (24). He, apparently, was cognizant only of the uredinial stage, for, in describing the fungus, he named it *Uredo glumarum*. Eriksson and Henning (6), in 1894, having discovered the telial stage, reclassified the organism and named it *Puccinia glumarum*.

Believing the non-existence of stripe rust in the United States east of the 103rd meridian might be explained on a basis of specific and inherent sensitiveness of the organism to environmental factors rather than its host relationships, the writers have sought to determine the effect of a partially controlled environment on the longevity and germination of both urediniospores and teliospores. It seemed important to know more concerning their viability and longevity under controlled conditions, not only as an effort to explain the absence of stripe rust in the Central and Eastern States, but also because of the application of the results to future research on this rust. A study of the germination of the teliospores was undertaken primarily to ascertain the optimum conditions for their germination and the development of the sporidia. This phase of the investigation was deemed preliminary and necessary to the proper carrying out of any organized investigation of the relation of *Puccinia glumarum* to a possible alternate host. The discovery of such a host may also throw some

[1] Cooperative investigations between the Idaho Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. Approved for publication by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 63.

[2] The writers wish to express their appreciation to Dr. C. W. Hungerford, Plant Pathologist of the Idaho Agricultural Experiment Station, for valuable suggestions during the progress of these investigations, and to Dr. H. B. Humphrey, Division of Cereal Crops and Diseases, United States Department of Agriculture, for criticisms of the manuscript during its preparation.

much-needed light on the question of the present distribution of stripe rust in North America. This alternate host, if there be one, may be adapted only to climatic and soil conditions more or less peculiar to the Pacific and Intermountain States. These investigations included, in addition to the urediniospores of *P. glumarum*, those of *P. graminis* and *P. triticina*. The teliospore-germination tests were confined solely to teliospores of *P. glumarum*.

Stripe rust has long been recognized as one of the most destructive cereal diseases in Europe, its importance there being comparable to that of bunt of wheat in the United States. Reports from the major wheat areas of Argentina show the occurrence of stripe rust in moderate to severe epiphytotics (14). Although stripe rust is known to occur in the United States only in the Pacific and Intermountain States, it is potentially very important to the major wheat-growing areas east of the Rocky Mountains where, by chance, it may some day be introduced by interstate crop transportation or from other parts of the world where stripe-rust epiphytotics commonly occur.

The severity of stripe rust in the Western Hemisphere is dependent largely on climatic conditions of the current crop year. In 1928 H. B. Humphrey of the United States Department of Agriculture and the senior writer inspected wheat fields in the Flathead Valley of Montana where stripe rust was present in severe epiphytotic form. Other observations, made in various sections of the Pacific Northwest, indicated no little damage from this rust.

In the apparent absence of the aecial stage of *Puccinia glumarum* there is sufficient evidence to show how the organism might overwinter. Eriksson and Henning (6) were of the opinion that the severity and extent of an epiphytotic were more directly dependent on hibernating mycelium than on surviving urediniospores. Hungerford (15), Rostrup (22), and Hecke (9, 10) agree that the mycelium is able to withstand adverse conditions. Although the evidence presented by Biffen (2) and Klebahn (16) is not so convincing, they claim that urediniospores play a part in the perpetuation of the fungus and may subsequently act as a source of infection. Hungerford (15), however, reports the finding of viable urediniospores during the months of September, 1917, to July, 1918, in western Oregon.

GERMINATION OF UREDINIOSPORES

Historical: Information concerning the germinability and longevity of urediniospores of *Puccinia glumarum* is very meager. Early European data are limited and confusing. Always there is a question regarding the identity of the particular species of the rust under consideration. Eriksson and Henning (6), quoting the data of earlier investigators or that of

their contemporaries, state that the germination of urediniospores lasts from 2–3 days (Wolff, 1875) to 8 months and 12 days (Barclay, 1891). Eriksson (7), himself, secured good germination of urediniospores of *P. glumarum* after cooling them for some time.

Probably the most accurate data available are those recorded by Hungerford (15), who states that "germination tests with urediniospores of stripe rust show that when the leaves of the infected host are kept in herbarium packets at ordinary room temperature the spores may remain viable at least 58 days. Urediniospores on leaves of wheat kept in open vials in a desiccator gave a slight percentage of germination at the end of 63 days. Urediniospores taken from wheat leaves, placed on glass slides and kept in a protected place in the laboratory, gave a trace of germination in 23 days."

Spore-Germination Technique: Melhus and Durrell (18) obtained a higher percentage of germination of urediniospores of *Puccinia coronata* in distilled or redistilled water than they did in tap water. Tap water at Ames, Iowa, was distinctly toxic to the urediniospores of *P. coronata.*

In an effort to discover a satisfactory method of germinating the urediniospores of *Puccinia glumarum* for subsequent use in spore viability studies at Moscow, some experiments involving the employment of ordinary tap water, distilled water, and rain water were conducted. In the preliminary attempts the percentage of germination in tap water was higher than in distilled water. In these tests uredinial material was stored at different temperatures. Two spore cultures were made for each temperature; one in distilled water, the other in tap water. These cultures were made merely by scraping the spores from the leaves into a drop of water on a glass slide and covering them with a cover-glass. The slides were placed in Petri dishes lined with moistened filter-paper and then placed in the refrigerator. No attempt was made to culture spores of the same age. In view of the fact that absence of free air inhibited germination, the cover-

TABLE 1.—*Comparative effect of distilled and tap water on the germination of urediniospores of Puccinia glumarum at Moscow, Idaho*

Storage temperature ° C.	Number of trials	Percentage of germination in distilled water	Percentage of germination in tap water	Number of spores counted
3	2	10.2	14.7	2,000
12	2	18.0	24.6	2,000
15	2	14.2	19.4	2,000
22	2	10.8	15.3	2,000
30	2	6.0	8.7	2,000

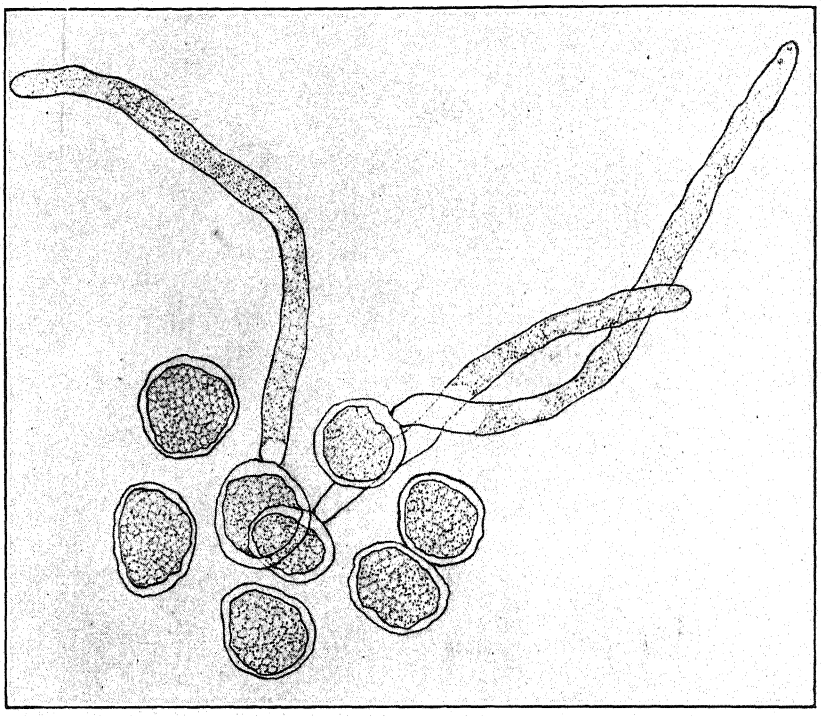


FIG. 1. A camera-lucida drawing of germinating urediniospores of *Puccinia glumarum*. These germinating spores are representative of those described as being kept in an air-tight chamber (Fig. 2) containing a relative humidity of 49 per cent and a temperature of 9° to 13° C. ×1,250.

glass was discarded in subsequent tests. Table 1 and figure 1 show the effect of the medium on germination and results obtained in the first attempts to germinate urediniospores.

Subsequent attempts to germinate urediniospores in rain water and in tap water gave 9.8 per cent germination in the one and 35 per cent in the other. A total of 4,000 spores was counted in determining these percentages.

Longevity of urediniospores: Peltier (20) states that the highest percentage of germination and the longest viability period of the urediniospores of form 3 of *Puccinia graminis tritici* occurred at a medium relative humidity. The results Hungerford (15) obtained with the urediniospores of *P. glumarum* have already been quoted. Hart (8) states that the optimum temperature for urediniospore germination of *Melampsora lini* (Pers.) Lev. is about 18° C. Fairly abundant germination obtains within the range of 6° to 23° C. The minimum lies near 0° C. and the maximum

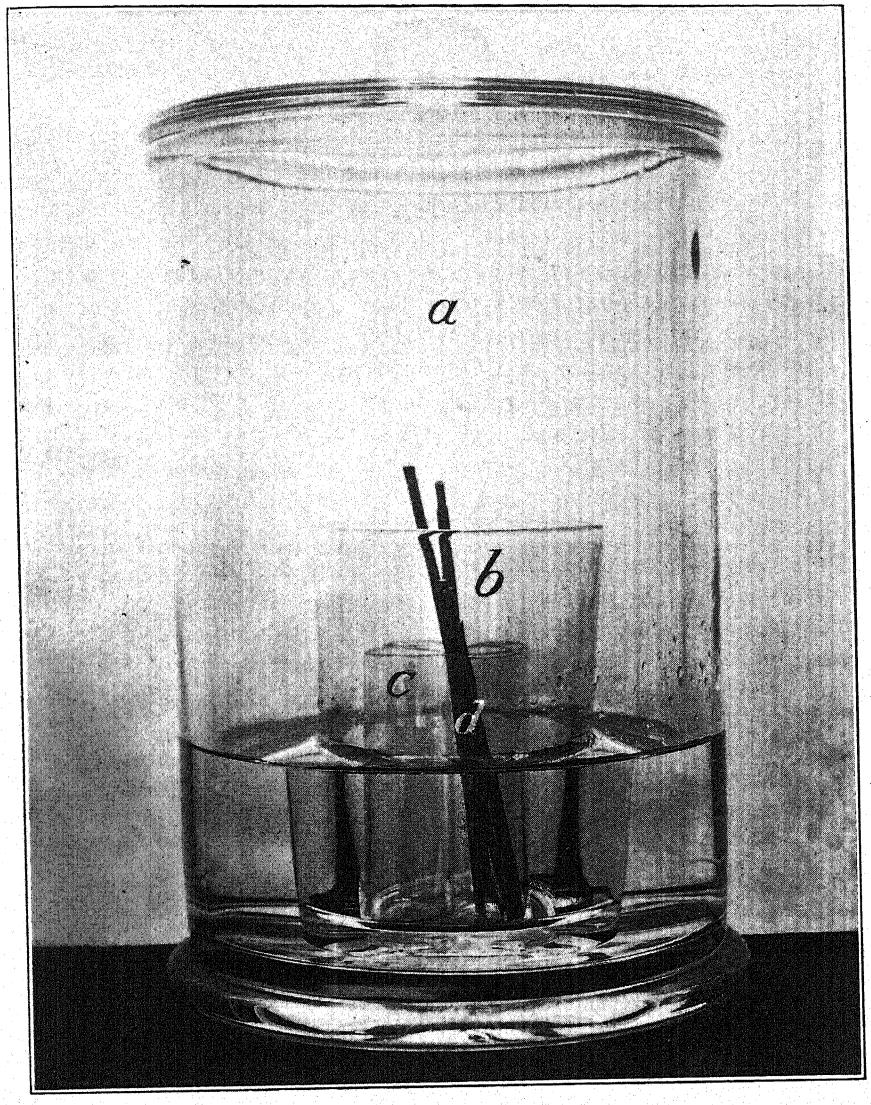


FIG. 2. *a*, A large glass chamber with a ground-glass top, containing a solution of sulphuric acid and distilled water of the desired concentration to create the necessary humidity; *b*, an ordinary glass tumbler in which are placed four glass vials; *c*, four glass vials, receptacles for the spore-bearing material; *d*, wheat-culm sections, placed in the vial to show arrangement of the spore-bearing material.

temperature is about 26° C. Under favorable conditions these spores retain their viability almost 3 months. They lose it more rapidly at high than at low temperatures. They also retain it longer at relative humidities of 40 and 60 per cent than at 20 and 80 per cent.

In attempting to determine the effect of various humidities on longevity air-tight chambers (Fig. 2) containing various strengths of sulphuric acid were employed. Humidities were obtained according to Stevens (24). Tumblers containing small vials in which the spore material was contained were placed in the various sulphuric-acid solutions. The whole was sealed with a greased ground-glass cover. The several temperatures were obtained in an ice room, refrigerator, storage basement, open laboratory, and an incubator. In addition to the urediniospores of *Puccinia glumarum*, those of *P. graminis phlei-pratensis* Eriks. and Henn. and *P. triticina* were used in 1927; and *P. graminis tritici* and *P. triticina* in 1928. Cultures were made in the manner described above; *i.e.*, the spores were placed in drops of tap water on glass slides which were placed in improvised Petri-dish moist chambers. Table 2 presents the results obtained from 2 years' study of the effect of temperature and humidity on the longevity of the urediniospores of the four rusts listed above.

In examining the results presented in table 2 it will be noticed that, under the conditions of the experiment, the urediniospores of *Puccinia glumarum* are not so long-lived as are those of the other three rusts. The spores of *P. glumarum* remained viable 88 days at a relative humidity of 49 per cent and a temperature of 9° to 13° C. Those of *P. graminis phlei-*

TABLE 2.—*The effect of various temperatures and relative humidities on the longevity of urediniospores of Puccinia glumarum, P. graminis phlei-pratensis, P. graminis tritici, and P. triticina, at Moscow, Idaho, in 1927 and 1928*

Relative humidity	OVEN							
	29° to 30° C. 1927 and 1928							
	Puccinia glumarum				*P. triticina*		*P. graminis phlei-pratensis*	*P. graminis tritici*
	Loose spores		Spores on leaf					
	Number of days urediniospores remained viable							
	1927	1928	1927	1928	1927	1928	1927	1928
100	Mold	Mold	Mold	Mold	Mold	Mold	Mold	Mold
76	6	21	0	8	30	32	9	23
49	6	28	11	35	32	35	26	32
25	0	14	5	25	20	25	26	28
1.5	0	25	0	23	4	18	21	18
Control[a]			14	16				

	DARK ROOM							
	13° to 26° C. 1927—23° to 26° C. 1928							
	1927	1928	1927	1928	1927	1928	1927	1928
100	3	Mold	Mold	Mold	4	Mold	2	Mold
76	10	36	20	32	21	32	28	25
49	8	18	44	42	33	35	28	52
25	6	18	27	18	30	25	38	28
1.5	0	18	3	11	16	21	26	34
Control			31	25				

	REFRIGERATOR							
	9° to 13° C. 1927 and 1928							
	1927	1928	1927	1928	1927	1928	1927	1928
100	14	11	4	11	8	4	7	5
76	16	53	51	69	75	104	111	124
49	12	60	75	*88*	107	118	*120*	*128*
25	10	53	35	63	77	110	117	114
1.5	0	52	30	56	36	71	117	62
Control			31	45				

	BASEMENT							
	9° to 21° C. 1927—3° to 11° C. 1928							
	1927	1928	1927	1928	1927	1928	1927	1928
100	14	4	4	4	3	6	15	5
76	16	57	44	68	63	118	69	120
49	12	60	53	78	88	*124*	88	126
25	12	48	33	60	69	105	76	110
1.5	0	39	33	41	42	71	62	73
Control			16	22				

	ICE ROOM							
	−2° to 5° C. 1927 and 1928							
	1927	1928	1927	1928	1927	1928	1927	1928
100	5	4	2	8	11	8	7	5
76	12	23	14	48	21	49	9	35
49	10	27	15	48	17	44	26	42
25	6	12	6	13	19	32	16	35
1.5	0	0	4	12	11	8	26	8
Control			7	38				

[a] Material in packets, held at the various temperatures, outside the humidity chambers.

pratensis and *P. graminis tritici* under the same conditions remained viable 120 and 128 days, respectively. Urediniospores of *P. triticina* remained viable 124 days in a relative humidity of 49 per cent and at a temperature of 3° to 11° C.

It will be noted also that a considerable difference in results was obtained in the 2 years' tests. In nearly every case the period of viability of each rust was longer in 1928 than in 1927. Two factors may have been responsible for this. First, the age of the spores at the beginning of the tests may have influenced the results. Second, the temperatures were held more constant in 1928 than in 1927.

In 1928, when those spores, held at a relative humidity of 49 per cent and at a temperature of 29° to 30° C., had ceased germinating, they were subjected to temperatures of 9° to 10° C. without removing them from the original humidity chambers. Cultures were made after the spores had been exposed to the latter temperature for 48 hours. The urediniospores of all three rusts resumed germination. Those of *Puccinia glumarum* germinated throughout 6 more days; those of *P. triticina,* 8 more; and those of *P. graminis tritici,* 11 more days. The spores in the 49 per cent humidity chambers, held at near zero C., also were transferred to room temperature (23° to 26° C.), after the spores had ceased germination at the original temperatures. After 48 hours' exposure, germination was resumed. The spores of *P. glumarum* germinated for 4 more days; those of *P. triticina,* 6 days; and those of *P. graminis tritici,* 6 days.

GERMINATION OF TELIOSPORES

Historical: Eriksson and Henning (6) were undoubtedly the first to report the germination of the teliospores of *Puccinia glumarum.* Prior to their investigations, according to them, it was commonly believed that the teliospores of the collective species *P. rubigo-vera* germinated only after a resting period, *i.e.,* during the spring following the season of their formation. These investigators, having separated *P. rubigo-vera* into several species, discovered that the teliospores of *P. glumarum,* unlike those of *P. rubigo-vera,* germinated without having to pass through a dormant period. Not all of the spores germinated in the fall. Eriksson and Henning (6) observed that a small percentage of teliospores of *P. glumarum* would germinate immediately after they had formed, while others apparently laid dormant until the following spring or fall. This, however, is not an unusual phenomenon in the genus Puccinia, for the spores of *P. dispersa* Eriks. and Henn. (17) possess the same ability. Other cereal rusts, such as *P. graminis* (7), *P. coronata* Corda (11), and *P. triticina* (17), produce teliospores that require a resting period before they will germinate.

Very little has been done to determine the viability of teliospores of *Puccinia glumarum* as well as the optimum conditions for their germination, together with sporidial formation. In view of these facts, a rather comprehensive study of this phase of the problem was undertaken. Such a study was intended to give definite data on the optimum conditions for germination of teliospores and sporidial formation and to serve as a preliminary procedure necessary to any proper investigation of the relation of *P. glumarum* to possible alternate hosts.

Preliminary teliospore-germination tests: Teliospores of *Puccinia glumarum* were first germinated, after a number of preliminary trials, in the fall of 1926. Material was collected on September 5, 1926, and tested the same week merely by placing the spores in tap water on a glass slide in a Petri dish lined with moistened filter-paper. This improvised moist chamber was then placed in the refrigerator. The spores were taken from *Hordeum jubatum* L. The percentage of germination was not determined. This method differed somewhat from that of Eriksson and Henning (6), for they did not remove the spores from the host matrix but permitted them to remain intact. The spore-bearing material was cut into small pieces and placed in water or in contact with moist soil.

Because of the response obtained with the spores from *Hordeum jubatum,* as noted above, another preliminary test was made with teliospores from *Cinna latifolia* (Trev.) Griseb. This specimen was collected on September 12, 1926. The spores were tested the following day and germinated within 2 days. The percentage of germination was again undetermined. In neither of the above cases would the percentage of germination have exceeded 5 per cent.

In an endeavor to determine the course of the viability of teliospores the senior writer collected telial material of *Hordeum jubatum* in the grass garden at Moscow, Idaho, on September 21, 1926. As is generally the case, the spores on this host developed on the leaf sheath, and this facilitated the experimental use of the material. The latter was divided into two groups and stored as follows: Group 1—in refrigerator; group 2—in incubator (28° C.).

Periodic germination tests were made with this material beginning September 21, 1926. On September 27, 1926, cultures were made from the rust-infected *Hordeum jubatum* collected September 5, 1926, and that collected September 21, 1926. In both cases the percentage of spores germinating was equivalent to that in the original trials.

Table 3 gives in detail the dates on which cultures were started and results obtained with groups 1 and 2, noted above. The same spore-culture procedure as that described in the foregoing was followed throughout the

studies here reported. Germination of the spores apparently had ceased by February, 1927. There were, therefore, no further cultures made except after the material was treated as described below.

On October 4, at the time spore cultures of groups 1 and 2 were made, a culture of teliospores was made from infected Jones Fife wheat collected by W. L. Popham at Bozeman, Montana, on August 20. The specimen from which the culture was made had been kept in the laboratory from the time it was received. At the end of 24 hours germination resulted only with the spores from groups 1 and 2. Of these, the spores stored in the incubator showed the highest percentage of germination. At the end of 72 hours a few spores from the wheat plant had germinated and there was an increase in the number of spores germinating in the other two cultures.

TABLE 3.—*Germination of teliospores of Puccinia glumarum that had been stored at different temperatures at Moscow, Idaho, in 1926 and 1927*

Date of starting culture	Group No.	Germination results				
		24 hours	48 hours	72 hours	96 hours	120 hours
1926						
Oct. 4	1[a]	+	Increase	Increase		
	2[b]	+	"	"		
Oct. 25	1	0	+			
	2	0	+			
Nov. 2	1	0	+	Same		
	2	0	0	0		
Nov. 8	1	0	+	Same	Same	
	2	0	0	0	0	0
Nov. 15	1	0	+	Same	Same	
	2	0	Slight	Same	Same	
Nov. 22	1	0	0		0	0
	2	0	0		0	0
Nov. 29	1	0	Slight			
	2	0	"			
Dec. 7	1	0	+			
	2	0	0			
Dec. 15	1	0	+			
	2	0	0			
Dec. 30	1	0	0		Few	
	2	0	0		"	
1927						
Jan. 10	1	0	0			
	2	0	0			
Feb. 28	1	0	0		0	0
	2	0	0		0	0

[a] Kept in refrigerator.

[b] Kept in incubator (28° C.).

Cultures from groups 1 and 2 were made on October 25. Examination of the cultures at the end of 48 hours showed germination in both, a greater number of spores germinating in that culture taken from the material stored in the incubator.

Germination tests of groups 1 and 2 were again started on November 2, 1926. At the end of 48 hours no spores had germinated in the culture from the material stored in the incubator. Good germination was obtained in the culture from the material stored in the refrigerator; in fact, it compared favorably with that of the various cultures made when the experiment was started. At the end of 72 hours neither culture from the incubator-stored material showed any germination. Germination in the culture from the refrigerator-stored material remained the same as at the end of the 48-hour period.

The fact that there was no germination in group 2 at the end of the 72-hour period and that there was the usual amount in group 1 at the end of 48 hours, notwithstanding the fact that previously there was a higher percentage among the spores of group 2, leads one to believe that such differences may have been due to differences in spore age. The spores tested were never taken from the same two culms twice in succession. When enough were obtained and mounted on a slide, the culm from which the spores were taken was discarded and a new culm from each group was used each time a test was made. Accordingly, each time a test was started spores were used and compared that might have varied in maturity. On the other hand, this difference in response shown by spores stored under two differing sets of conditions might have been directly due to this difference in environment.

The walls of many of the spores seemed quite impervious to water and there was no consequent swelling of the spores. They appeared to be of a healthy yellow color and normal cell content. Often one cell of a teliospore would increase in size, indicating the absorption of water, while the other would still retain its normal size and appearance. All spores that germinated were larger than those that did not.

On November 8, 1926, the cultures of groups 1 and 2, begun on November 2, were exposed and allowed to dry 24 hours. At the end of this time water was again added to the slides and the moist chambers and they were again placed in the refrigerator. No spores had germinated at the end of 24 hours. New cultures of groups 1 and 2 were started on November 8. At the end of 48 hours germination was noticed only in the culture from group 1.

The two cultures of November 2, allowed to dry for 24 hours, beginning November 8, showed no germination at the end of 72 hours. They were again exposed and allowed to dry.

When cultures of November 8 were examined, 96 hours later, germination was observed only in the culture from group 1. In this culture the percentage of germination of spores suspended separately in the water was quite negligible. The greatest amount of germination was noticed in the spore aggregates still adhering to portions of the host. Groups of spores free from any host matrix showed little, if any, germination. At the end of 120 hours no differences were noticed in the culture of November 8.

The cultures of November 2, dried on November 8, moistened November 9, and dried November 12 were again moistened and placed in the refrigerator on November 13. Forty-eight hours later none had germinated.

On November 15, 1926, cultures were again made of groups 1 and 2, also of teliospores from infected barley C. I. 1315, collected in the greenhouse on November 13, 1926. Of the above, a few spores of group 2 had germinated at the end of 48 hours, more from group 1, and none from the barley. At the end of 72 hours some of the spores from the barley had germinated. No change was observed in the cultures from groups 1 and 2. At the end of 96 hours more spores from the barley had germinated.

Groups 1 and 2 were again cultured on November 22, 1926. No germination was observed at the end of 96 hours, so the cultures were discarded. The culture from barley started November 15 still showed viable spores. Many of the older germ tubes had lengthened and ramified. The contents of the germinating cell of the spore was generally to be found in the growing tip of the promycelium and was distinctly granular, an observation which conforms to the finding of Eriksson and Henning (6). Cultures of groups 1 and 2, made November 29, 1926, each, contained a few germinating spores at the end of 48 hours. A culture from the barley collected in the greenhouse November 13, made on November 29, failed to respond at the end of 48 hours.

It was noticeable that the number of teliospores responding had gradually decreased. None of the more recent cultures showed the amount of germination that had been obtained with the same material in September.

Cultures were again made on December 7, 1926, from groups 1 and 2 and from barley C. I. 1315, collected in the greenhouse on November 13. No spores had germinated in any of these cultures at the end of 48 hours.

Cultures of groups 1 and 2, started December 15, 1926, showed germination only in the case of group 1 at the end of 48 hours. A culture from *Hordeum jubatum,* collected December 3 and started December 15, showed no response at the end of 48 hours.

On December 18, 1926, teliospores were collected on *Hordeum jubatum,* in the grass garden at Moscow, and cultured the same day. At the end of 48 hours none of the spores had germinated.

Groups 1 and 2 were again cultured on December 30, 1926. A few spores of each group had germinated at the end of 96 hours.

To test the germinability of old teliospores that had been stored in the laboratory from date of collection, cultures were made of spores collected at Moscow on *Agropyron cristatum* (L.) Gaertn., in the summer of 1920, and of teliospores collected at Moscow on *Hordeum jubatum* July 1, 1925. No germination was noticed in either group of cultures at the end of 72 hours.

Groups 1 and 2 were again cultured on January 10, 1927. No spores had germinated in either culture at the end of 48 hours.

Teliospores collected on January 28, 1927, on barley C. I. 1315, grown in the greenhouse, were cultured January 31 in the usual manner. No spores had germinated at the end of 48 hours nor at the end of 72 hours. Within a week a few spores had germinated and further germinations occurred until the end of the eleventh day, when the culture was discarded.

On February 28, 1927, cultures of both groups 1 and 2 were made. At the end of 5 days no germination was observed in either culture. Most of the spores on the slide from group 1 appeared abnormal, in that they lacked the normal yellowish brown color, and many seemed devoid of the characteristic granular contents. The spores on the slide from group 2 were normal in every respect.

The reaction of those teliospores that germinated and failed to produce sporidia was in all probability due to faulty technique. A higher percentage of germination occurred near the edge of the cover-glass where air content of the water was greatest. Those spores that did germinate nearer the center of the cover-glass produced longer and more tortuous germ tubes. These results closely approximated those of Melhus, Durrell, and Kirby (19) who, in working with the teliospores of *Puccinia graminis*, state that a relative humidity of at least 95.6 per cent is required for teliospore germination and the production of sporidia. In a humidity of 100 per cent sporidia are more profuse. Teliospores submerged in a drop of water produce long attenuated germ tubes and sporidia production is slight or wanting. Subsequent tests indicated that the cover slip placed over each culture produced conditions that inhibited germination. Cultures in which the cover slip was absent gave a higher percentage of germination and a profuse production of sporidia.

Effect of various stimuli on the germination of teliospores: For some time previous to the date of the last attempts to germinate the spores, February 28, 1927, it was noticed that those spores that had been stored in the refrigerator remained viable longest. But, even in this group, the percentage of germination was very small. Because of this and the fact that the cultures showed so many water-impervious spores, it was proposed to

treat them in various ways in an attempt to penetrate the impervious cell walls, if such they be, and thus stimulate such spores to germinate or possibly revive this capacity in others.

Chemical stimuli: Acids: Thiel and Weiss (25), working with the teliospores of *Puccinia graminis tritici,* induced them to germinate prematurely by exposing them to a 1 per cent solution of citric acid for 15 minutes. Durrell (5) stimulated the spores of *Basisporium gallarum* Moll. to germinate by exposing water-drop cultures of spores to CO_2 in concentrations of from 1 to 5 per cent. Howe (12) secured the highest percentage of germination with the spores of *Ustilago levis* (K. and S.) Mag. in a medium having a pH of 4.9 secured by a continuous flow of CO_2 into the spore suspension.

In the light of these investigations it was decided to expose teliospores of *Puccinia glumarum* to a 1 per cent solution of citric acid. On March 11, 1927, teliospores of group 1 were treated with a 1 per cent solution of this acid for 15 and 30 minutes, respectively. This was accomplished by submerging in the acid spore-bearing portions of the host culm. After removing from the acid and rinsing thoroughly in tap water, the spores were cultured in the usual manner.

In less than 24 hours excellent germination was obtained with the spores which had been exposed to the acid for 15 minutes. A few spores had germinated in the other culture. At the end of 72 hours the number of spores germinating in the culture which had been exposed to the acid for 15 minutes gave the highest percentage of germination so far secured. Increased germination had resulted in the other culture but it was not so great as in the culture exposed to the acid for 15 minutes. This difference may be due not to the difference in treatment but to the fact that the spores used for the two cultures were obtained from different culms. Other conditions, therefore, such as difference in age of spore, thickness of spore wall, thickness of host epidermis covering the spores, etc., may have affected the germination of the spores.

In view of the reaction obtained with citric acid a number of other acids were used in 1 per cent concentrations. Only the spores from group 1 were used in these tests. The acids employed and results obtained with them are given in table 4.

It may be that the stimulating effect, if it could be called such, is due to the hydrogen-ion concentration rather than to the particular acid used. For example, the normalcy of the citric acid, which was a 1 per cent solution, was approximately .1/N, with an approximate pH value of 2.26. The hydrochloric acid was a 1 per cent solution of the commercial-concentrated product. It was about .1/N, with a pH value of 1.065. The use of the citric acid resulted in germination; that of the hy-

TABLE 4.—*The effect of various acids on the germination of teliospores of Puccinia glumarum on a single culm*

Treatment	Exposure	Germination results	
		24 hours	48 hours
Check. No treatment		0	3 spores
Boric acid, 1 per cent	15 min.	Good	Same
Hydrochloric, 1 per cent of commercial concentrated	"	0	Slight
Nitric, 1 per cent of commercial concentrated	"	0	Very good
Check. No treatment		0	0
Chromic, 1 per cent	"	0	Slight
Oxalic, 1 per cent	"	0	4 spores
Check. No treatment	"	0	Slight
Sulphuric, 1 per cent of commercial concentrated	"	Slight	"
Lactic, 1 per cent	"	"	"
Acetic, 1 per cent	"	"	Good

drochloric acid did not. The latter was, therefore, further diluted and spores were exposed to it for 15 minutes on March 18, 1927. Within 24 hours the spores thus exposed showed good germination, while only two germinating spores were noticed on the check slide. Within 48 hours the percentage of germination of the treated spores had materially increased. No increase in the number of germinating spores was noticed on the control slide.

On March 30, 1927, the same treatments were administered to the teliospores of group 2. The same procedure was followed in exposing the spores to the acids and the same culture technique was employed as in the preceding experiment. No germination resulted in any of the cultures.

On April 11, 1927, the spores of group 1 were again given the acid treatment for a period of 20 minutes instead of 15. The cultures were examined at the end of 24 and 48 hours and at the end of 1 week. In no case was there any germination.

The pH values of the various acids used ranged from 6.9 to 1.6, as determined with a La Motte No. 4 B Hydrogen-ion Testing Set.

Teliospores of *Puccinia glumarum* on *Aegilops cylindrica* Host. were first collected on July 15, 1927. Attempts to germinate them failed.

Fresh teliospore material was collected at Moscow on *Hordeum jubatum* on July 28, 1927. All attempts to germinate the spores resulted negatively. The effect of acids was again tested and, in addition to those named in the foregoing, the following organic acids were used: Tartaric, pH 2.1; maleic, pH 2.6; succinic, pH 3.3; salicylic, pH 3.7; phthalic, pH 2.7; and benzoic, pH 3.5. None of them induced germination.

Material collected July 28, 1927, was treated on August 23, 1927, with the various acids listed in table 3 for 15 minutes in the manner heretofore described; then they were cultured in the usual manner. One set of cultures was placed in the refrigerator, while a second set was allowed to remain in the laboratory. No germination was obtained in any of the trials.

Material stored in the ice box from date of its collection, August 2, 1927, was exposed to various dilutions of citric, oxalic, sulphuric, nitric, and hydrochloric acids on February 8, 1928. The spore-bearing culms of the host were immersed in the acids for intervals varying from 1 minute to 20 minutes, depending upon the concentration of the acid. The material was immersed in the weaker solution a longer period than in the more concentrated solutions. Nontreated controls were run for each acid. No germination was secured in any case. From the appearance of some of the spores after a week's incubation, partricularly those treated with the more concentrated solutions, it was apparent that the treatment was too severe.

Other chemicals: Rosa (21) obtained a mild stimulating effect on dormant potato tubers with ethylene in concentrations of from 1:400 to 1:2000 of air. He also found that solutions of 2 to 3 per cent sodium thiocyanate and ammonium thiocyanate were toxic and that ethyl bromide and ethylene dichloride hastened sprouting.

On November 20, 1927, material collected in July and held in the refrigerator was treated with ammonium thiocyanate pH 4.6, ethyl bromide pH 4.5, and ethylene dichloride pH 4.6, for 30, 60, and 120 minutes. Cultures from each treatment were made but gave only negative results. These treatments also were used on material that had been stored outside, but no germination was secured.

Teliospores, still adhering to the culms of the host, were placed in an air-tight chamber containing equal amounts of pure oxygen and ethylene gas. They were permitted to remain in this mixture of gases for 48 hours at room temperature. At the end of the 48-hour exposure the spores were cultured in the usual manner and incubated in the ice box for 72 hours. Six spores in the culture germinated, exhibiting a stronger and healthier germ tube than had been secured heretofore. A second trial gave negative results. The material used above was part of that collected July 28, 1927, and stored in the ice box from the date of collection.

As stated in the foregoing, it has been observed that when teliospores do germinate, they show a stronger and higher percentage of germination when attached to portions of the host or near the outer edges of the cover slip. It was assumed that this difference might be due to the more abundant oxygen in such localities. Durrell (5) noticed this same difference in working with *Basisporium gallarum*. Accordingly, hydrogen peroxide in 4 concentrations was employed in which teliospores were immersed for 15

minutes and then cultured in the same solution. Five, 10, 15, and 20 per cent solutions of the chemical were used. After incubating for 1 week in the ice box, readings were made. No effect was observed on the percentage of germination of the spores.

Exposing teliospores still adhering to the culms of the host to ether contained in desiccators had no stimulating effect upon germination. The spores were exposed to this gas for 12, 24, 48, and 72 hours, respectively, by placing them in the desiccators containing vials of sulphuric ether.

PHYSICAL STIMULI

Low temperatures: On October 14, 1927, telial material, collected the previous July and stored in the refrigerator since collection, was placed out of doors. The material was contained in a small gauze sack and hung from an east window. Up to January 16, 1928, cultures were made every 2 weeks. None, however, showed germination. At the time the material was placed outside the percentage of germination of the spores was 3. The temperature during this period ranged from 78° F. on October 20 to −17° F. on December 17.

One might conclude from the above results that, unlike the teliospores of *Puccinia graminis,* which will germinate only when subjected to all changes of winter weather (4), those of *P. glumarum* are not in the least affected by such conditions. Later investigations, however, seem to contradict such a presumption.

Parallel with the biweekly tests made with the spores stored outside, the same type of cultures was made from the material still remaining in the refrigerator. These tests showed sporadic and unequal germination.

In the fall of 1928 fresh material was subjected to outdoor temperature in a manner similar to that employed in the winter months of 1927–28. From the results obtained there was an apparent stimulation. It will be noticed that with the tests of both years moisture was not considered. Being stored as it was, the material outside was never thoroughly wetted. It was held under air-dry conditions constantly.

Assuming that a combination of high humidity and freezing temperatrue might enhance germination, material was exposed to such conditions. It was first thoroughly wetted and placed between moistened filter-paper. Then it was exposed 5 days to a temperature ranging from −2° C. to −5° C. At the end of this period the spores were cultured in the usual manner.

Table 5 gives the results of these tests, including the name of the host, date and place of collection, and number of replications. It is quite evident that freezing alone does not induce teliospore germination. Moisture and low temperature combined seem necessary (Fig. 3).

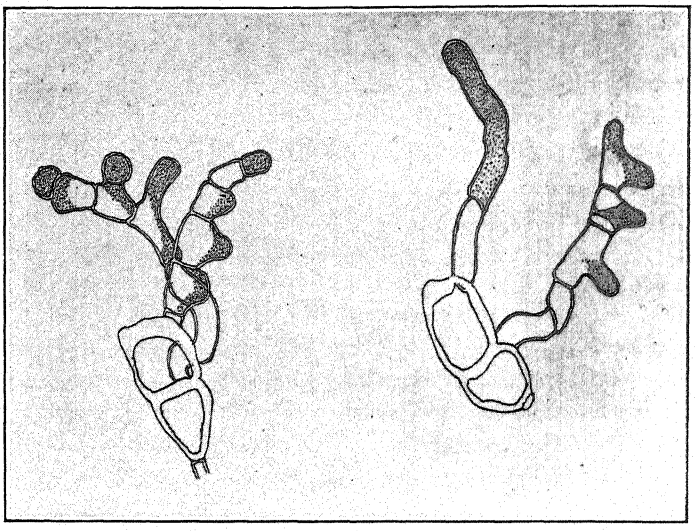


FIG. 3. A camera-lucida drawing of germinating teliospores of *Puccinia glumarum*. These teliospores were germinated in distilled water on open glass slides. The slides were placed in a moist chamber and allowed to remain in ice box for 48 hours. The drawings were made at the duration of that time. Drawings × 1,000.

Alternate wetting and drying: On November 17, 1927, spores still on the culms of *Hordeum jubatum* which had been stored in the refrigerator since July 28 and under outdoor conditions since October 14 were immersed in water for 24 hours. They were then removed and allowed to dry for 24 hours and again immersed for another 24 hours. When dry cultures were made and incubated in the refrigerator for 1 week, no germination resulted.

Alternating temperatures: Teliospores on culms of *Hordeum jubatum*, stored in the refrigerator, were transferred to an oven with the temperature at 30° C. They were left in the oven for 24 hours, then cultured in the usual manner and incubated 7 days in the refrigerator. About 3 per cent of the spores had germinated at the end of this time. Another lot of teliospores, placed in the oven at 30° C. for 48 hours, were then moved to the refrigerator for 24 hours. No germination resulted from this procedure. By another method cultures of spores were held in the refrigerator for 24 hours, then at room temperature for 48 hours. No germination was observed.

DISCUSSION AND CONCLUSIONS

From the results obtained by Becker (1), who found that urediniospores of *Puccinia glumarum* had the ability to survive 433 days at 0° C. in a relative humidity of 40 per cent, it might follow that she studied a physiologic

TABLE 5.—*Low temperature and high humidity effects on the germination of the teliospores of Puccinia glumarum at Moscow, Idaho, 1928–1929*

Treatment	Host	Date of collection	Place collected	Dates tested	Per cent germ.	Ave. per cent germ.
Frozen 5 days	*Hordeum jubatum*	Sept. 20, 1926	Grass garden Exp. Station, Moscow, Idaho	10– 1–28	0	
				10–10–28	0	
				10–20–28	0	0
				11– 5–28	0	
				1–21–29	0	
Check	"	"	"		0	0
Frozen 5 days	Jenkins Club wheat	July 20, 1927	Agronomy plots, Expt. Sta., Moscow, Idaho	10– 1–28	3	
				10–10–28	4	
				10–20–28	3	3
				11– 5–28	2	
				1–21–29	Tr.	
Check	"	"	"		0	0
Frozen 5 days	"	July 25, 1928	Agronomy plots, Expt. Sta., Moscow, Idaho	10 –1–28	15	
				10–10–28	20	
				10–20–28	11	14
				11– 5–28	10	
Check	"	"	"		0	0
Frozen 5 days	*Agropyron tenerum longifolium*	June 16, 1928	Grass garden Expt. Station, Moscow, Idaho	10– 1–28	40	
				10–10–28	30	
				10–20–28	20	29.6
				11– 5–28	50	
				1–21–29	8	
Check	"	"	"		0	0
Frozen 5 days	Jones Fife wheat	July 8, 1928	Kalispell, Montana	10– 1–28	62	
				10–10–28	30	
				10–20–28	35	
				11– 5–28	38	35
				1–21–29	15	
				3–20–29	30	
Check	"	"	"		3	3

form of the rust that produced spores capable of withstanding conditions much more adverse than the spores of our physiologic form of the rust. In other words, Becker's results and those here presented afford further evidence that the form or forms in the United States are unlike those present in Europe.

The evidence on which to explain the absence of *Puccinia glumarum* in the United States east of the 103rd meridian is insufficient. Further studies of such phases of the problem as the effect of environment on spore germination, infection, incubation, and subsequent sporulation, and alternate-host studies of unconnected aecial rusts must be carried on before definite conclusions can be drawn.

The teliospores of *Puccinia glumarum* can germinate immediately on maturity without first having to pass through a resting period or be subjected to alternate freezing and thawing. It is not assumed, however, that such conditions are negative in their effect, for freezing teliospores of the stripe-rust fungus in the presence of moisture either revives this ability in spores which seem to have lost it, or stimulates it in others that apparently have not reached the proper state of maturity. All of the spores do not germinate during the season in which they were formed, for some still possess this ability during the spring following the previous fall's collection.

In respect to their ability to germinate during the season of their formation, the teliospores of *Puccinia glumarum* are not unlike those of *P. dispersa*. This characteristic seems unusual, for the teliospores of the more commonly known rusts, *P. graminis, P. coronata,* and *P. triticina,* do not possess this ability, but, on the other hand, require a period of dormancy before germination.

This early germinating ability exhibited by the teliospores of *Puccinia glumarum,* and the fact that the stripe-rust fungus can withstand adverse conditions by means of resting mycelium bring to mind the possible relationship that might exist in respect to the lack of a known aecial host. Ability to hibernate by means of dormant mycelium might preclude the necessity of an alternate host. Having no need for such a host the teliospores may have gradually lost their important function in the completion of the life cycle of the fungus. In doing so, they may have gradually acquired the ability to germinate immediately upon formation, rather than pass through a resting period, and the possible accompanying adverse condition, to await optimum conditions for germination and the appearance of a susceptible aecial host. Proof of the existence of an aecial host has yet to be established. If it does exist, it plays an unimportant rôle in the perpetuation of the fungus.

Of the various stimuli employed in trying to revive or stimulate germination of the teliospores, freezing temperatures, combined with high

humidity, resulted in the most pronounced stimulation. Herein are the teliospores of *Puccinia glumarum* similar in reaction to those of *P. graminis* and *P. coronata*. The stimulation resulting from acids or other chemicals in various dilutions was neither consistent nor conclusive.

Abundant sporidia develop if the right method of teliospore germination is used. This being the case, it is logical to assume that any possible lack of an alternate or aecial stage of the rust is not due to failure to produce sporidia.

SUMMARY

Stripe rust has long been recognized as one of the most important cereal diseases in Europe. In Argentina it occurs in moderate to severe epiphytotics. In the United States, so far as known, it occurs only in the Pacific and Intermountain States and is there only moderately severe.

Attempts to germinate urediniospores of *Puccinia glumarum* in tap water, distilled water, and rain water resulted in the highest percentage of germination taking place in tap water.

Under the conditions of the experiment the optimum humidity and temperature for the retention of germinability of the urediniospores of *Puccinia glumarum* were 49 per cent and 9° to 13° C., respectively. Spores held under these optimum conditions remained viable 88 days.

Urediniospores of *Puccinia graminis phlei-pratensis* and *P. graminis tritici* remained germinable under the same conditions for 120 and 128 days, respectively. Those of *P. triticina* were viable 124 days at a temperature of 3° to 11° C. and a relative humidity of 49 per cent.

Transferring the spores which had been held in the 49 per cent humidity containers at a temperature of 29° to 30° C., after all germination had ceased, to temperatures of 9° to 10° C., for 48 hours resulted in the renewed germination of the spores of all three rusts. The spores of *Puccinia glumarum* germinated 6 days longer; those of *P. graminis tritici*, 11 days longer, and those of *P. triticina*, an additional 8 days. In like manner, the urediniospores held at freezing and a relative humidity of 49 per cent were transferred, after they had ceased germinating, to room temperature (23° to 26° C.) and kept there for 48 hours. Under these conditions the urediniospores of *P. glumarum* germinated 4 days longer; those of *P. graminis tritici* and *P. triticina* 6 days longer.

Germination of teliospores of *Puccinia glumarum* was first obtained by the senior writer in September, 1926, from newly collected material.

At temperatures prevalent in an ordinary ice refrigerator, teliospores stored under such conditions retained their germinability longer that simultaneously collected spores held in an oven at 28° to 30° C.

Teliospores incubated under these conditions apparently had lost their ability to germinate by the following spring, but were revived by various stimuli.

Many teliospores present in the cultures seemed to be impervious to water.

Tests indicated that an undetermined factor caused nonuniformity of germination in the cultures made from time to time. Indications were that this factor was difference in age of spores.

Various stimuli produced inconsistent results in an attempt to revive this power of the spores to germinate.

Immersing the spore-bearing material in a 1 per cent solution of citric acid for 15 minutes sufficed to induce the latent spores to germinate after they apparently had lost this ability.

Boric, hydrochloric, nitric, chromic, oxalic, sulphuric, lactic, and acetic acids, in 1 per cent or weaker solutions, seemed to stimulate germination.

Induced germination of teliospores was not the result of any certain pH value of the acids.

Fresh teliospores collected in 1927 did not possess the same germinating ability as did those collected in 1926.

Results obtained with ammonium thiocyanate, ethyl bromide, ethylene dichloride, ethylene-gas-and-oxygen mixture, and hydrogen peroxide were not conclusive in their ability to induce germination of teliospores.

Alternate wetting and drying of teliospores at ordinary temperatures did not induce germination, nor did freezing under air-dry conditions.

Exposing teliospores to a combination of high humidity and freezing temperature resulted in a marked increase in germination.

Abundant sporidia developed when the teliospores were germinated in an exposed drop of water on a glass slide.

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TRANSPIRATIONAL HISTORY AS A KEY TO THE NATURE OF WILTING IN THE FUSARIUM WILT OF PEAS[1]

MAURICE B. LINFORD

Among the hypotheses set forth by diverse investigators to account for the wilting characteristic of various Fusarium wilts, that which regards wilting as a result of failure of the water supply, by whatever agency this may be brought about, has frequently been mentioned. The frequently observed phenomenon of temporary wilting of affected plants during periods favorable for rapid transpiration is more readily explained by this than by other theories. In the Fusarium wilt of the pea (*Pisum sativum* L.) caused by *F. orthoceras* App. and Wr. var. *pisi* Linford[2] this alternate wilting and recovery has been seen only in large plants. In young plants it probably does not occur. When wilting begins it proceeds directly to the collapse and death of the wilting leaflets. Such sudden wilting may occur in plants grown in cotton-plugged culture tubes in indirect sunlight where the transpirational demand is slight, and, both in such tubes and in open pot culture, it is sometimes accompanied by a water-soaked appearance of collapsing parts. This wilting, coming as a sharp reaction from a condition of extreme turgidity which precedes it,[3] suggests that the loss of turgor may be not a consequence of an inadequate supply of water but rather a result of death of the cells and loss of their powers of retaining water.

Daily determinations of the transpiration rate of diseased plants during wilting should indicate which of these interpretations is the correct one. Accordingly, in the course of other investigations of the pea-wilt disease the following experiment was conducted to trace comparatively the transpirational histories of healthy plants growing in clean soil and of plants growing in wilt-infested soil throughout the entire course of the disease. Since it became impossible to repeat the experiment the data are not considered final. This report sets forth indicative evidence relative to the nature of wilting and suggests a mode of attack which may be made to yield highly important results.

[1] This paper is an offshoot of investigations of pathogenesis and resistance in the Fusarium wilt of peas conducted under a fellowship appointment, from the National Research Council, in the Department of Botany, University of Wisconsin. The writer gratefully acknowledges the helpful advice of Professor B. M. Duggar and the assistance of Mr. Oliver S. Orton in carrying on this work.

[2] Linford, M. B. A Fusarium wilt of peas in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 85. 1928.

[3] See footnote 2.

Peas of uniformly susceptible pure line of the Alcross variety were planted individually in cylindrical glass tumblers which measured about 6.5 cm. in diameter by 9.5 cm. in depth, each containing 250 gm. of soil previously adjusted to approximately 60 per cent of its water-holding capacity. Twenty were prepared with clean, uncooked soil, and 20 with wilt-infested soil that, 1 year before, had been sterilized and inoculated with a pure culture of the wilt fungus.

As soon as the young sprouts had emerged, 12 of the most uniform of each lot were selected for the experiment. The seedlings were removed from 10 tumblers used as checks on water loss. A layer of melted wax[4] was then poured over the soil in each tumbler, reducing the possible channels of escape of water to the plant and the narrow glass-funnel tube which extended to the bottom of each tumbler for the addition of water during the experiment. In the check tumblers, in which these funnels and imperfections in the wax seals were the only channels of egress, weighings indicated that the water loss during the experiment was negligible.

Both lots of soil were adjusted to 60 per cent of their water-holding capacities before planting, and, then, before pouring the wax seals, each tumbler was restored to its initial weight by the addition of water. For convenience in weighing throughout the experiment, all tumblers were then adjusted to equal weight within the limits of 1 gram by the addition of warm lead shot (BB) which melted itself into the surface of the wax. The tumblers were then spaced equally on a wire-mesh support, 15 inches above the table top, in a large glass room where exposure to light, heat, and circulation of air was uniform. The temperature fluctuated chiefly between 20° and 24° C., reaching the limits of 15° and 27° C. during the experiment. Daily records of water loss, of the development of plants, and of wilt symptoms were made individually for each plant. Water was added from a burette while the tumbler stood upon one pan of a long-arm balance, counterbalanced by the initial adjusted weight of the tumbler. Thus the daily water loss per plant was read directly from the burette to tenths of 1 cubic centimeter, as water was added to restore that lost daily. On the twelth day after the wax seal was poured, total surfaces were estimated for every plant separately based upon a series of measurements of every leaflet, stipule, and stem.

The 12 plants in clean soil made vigorous and uniform growth as indicated by the uniformity of total surface on the twelfth day, recorded in table 1. Two plants in infested soil were attacked by Rhizoctonia and

[4] The most satisfactory wax seal was the following, modified from the formula of Briggs and Shantz: paraffin (Parowax), 5 parts; white petrolatum, 2 parts; and beeswax, 2 parts. When cooled to near its melting point before being poured, this wax caused no visible injury to the delicate sprouts.

dwarfed so badly that they were discarded. The remaining 10 showed fair uniformity in their development and also in the development of disease. Positive symptoms were noted first on the fifth day in 2 plants, and, by the ninth day, all 10 were diseased. By the eighteenth day, at the close of the experiment, the leaves and stem tips of all 10 plants in infested soil were fully wilted.

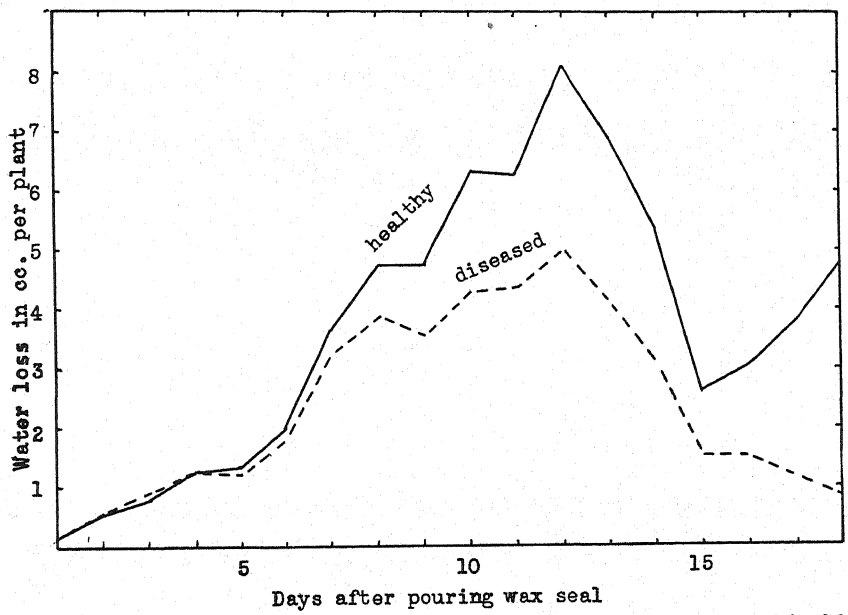


FIG. 1. Daily average loss of water, in cubic centimeters per plant, from 12 healthy and 10 diseased Alcross variety pea plants. First symptoms of the wilt disease appeared on the fifth day; and all leaves of all affected plants were fully collapsed on the eighteenth day.

With the progressive increase in transpiring surface of the healthy plants, the daily loss of water should have increased progressively, but, as shown in figure 1, this was not the case. A sharp reduction occurred in the transpiration of both healthy and diseased plants between the twelfth and the fifteenth days as the result of a cold, cloudy period. This figure shows, however, that the daily loss from plants in the infested soil was slightly higher than from those in clean soil during the opening days of the experiment but that it gradually fell off during the succeeding days as the disease developed. This decline was the result of at least two factors as illustrated by table 1. In the infested soil the plants were distinctly dwarfed. On the twelfth day, when the collapse of affected plants had scarcely begun, the average surface exposed by the diseased plants was only 75.4 sq. cm. as compared with 96.4 sq. cm. exposed by the healthy

TABLE 1.—*Total areas and rates of transpiration of healthy pea plants and of plants in medium stages of Fusarium wilt. Areas were computed from measurements of every leaflet, stipule, and stem. These data are for plants of a susceptible pure line of the Alcross variety, on the twelfth day after the beginning of the experiment*

Healthy plants in clean soil				Diseased plants in infested soil			
Plant number	Total area	Water loss per day		Plant number	Total area	Water loss per day	
		Per plant	Per square meter			Per plant	Per square meter
	sq. cm.	*cc.*	*cc.*		*sq. cm.*	*cc.*	*cc.*
C–1	98.0	9.1	929	W–1	93.9	7.0	746
C–2	84.7	7.4	874	W–2	52.9	3.1	586
C–4	95.6	7.7	806	W–8	78.0	5.8	744
C–5	95.1	9.3	978	W–9	72.7	4.7	647
C–8	95.5	7.6	796	W–10	88.9	6.3	709
C–9	90.3	8.4	931	W–12	83.0	5.8	699
C–11	87.1	7.7	884	W–14	86.5	5.6	648
C–13	116.4	8.1	696	W–17	67.8	4.5	664
C–14	102.8	7.4	720	W–20	63.1	3.8	602
C–15	99.4	8.0	805	W–21	67.3	3.7	550
C–19	99.1	8.5	858				
C–21	92.3	7.7	834				
Average	96.4	8.1	838	Average	75.4	5.0	667

plants. But this smaller surface was not the only cause of reduced water loss, for, while the diseased plants showed 78.2 per cent as much surface area as the healthy plants, they lost only 61.7 per cent as much water. Computed to the basis of water loss per square meter of surface per day, as shown in table 1, the water loss from the healthy plants was 838 cc. as compared with 667 cc. from the diseased plants. These figures thus indicate that prior to wilting the diseased plants had a lower rate of transpiration than the healthy.

The daily trend of transpiration with progress of the disease is obscured in these figures by the wide fluctuation produced by environmental conditions. The daily average loss of water from the healthy plants was therefore adopted as a standard of comparison, and a transpirational ratio, $\frac{\text{water lost by diseased plants}}{\text{average water lost by healthy plants}}$, was computed. This ratio is strictly relative and is independent of daily fluctuations in the environment in so far as diseased and healthy plants respond similarly to environmental conditions.

Figure 2 represents graphically the daily transpirational ratios of 4 individual affected plants and the average of all 10 affected plants. In agreement with figure 1, it shows a general decline after the opening days

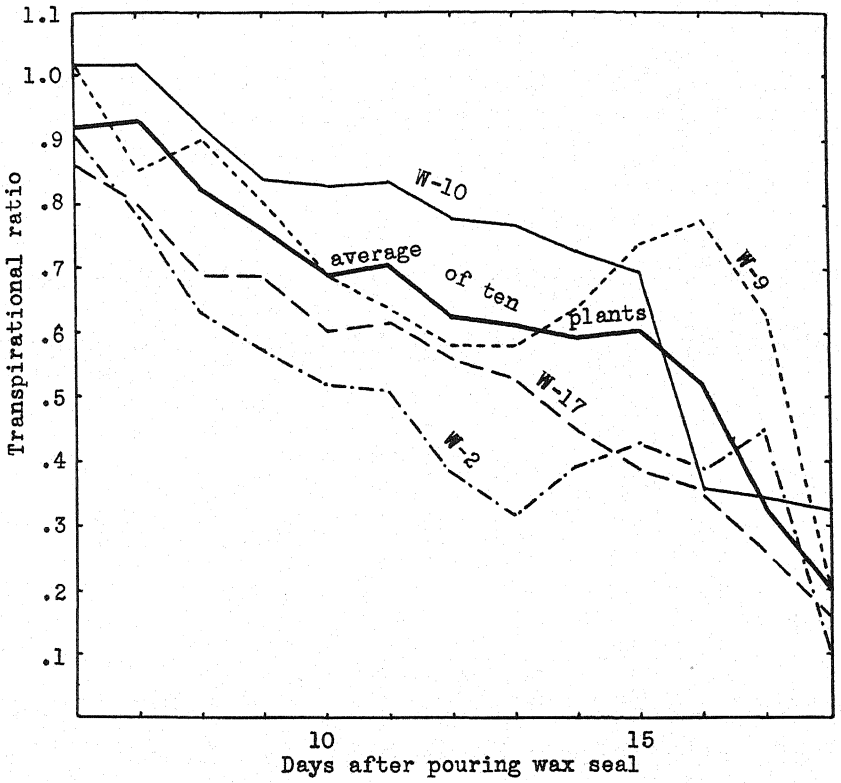


FIG. 2. Transpirational histories of pea plants affected with Fusarium wilt. Daily loss of water from 4 separate plants and average daily loss from 10 plants, expressed as the transpirational ratio; *i.e.*, $\frac{\text{water lost by diseased plants}}{\text{average water lost by healthy plants}}$. The 4 individual plant curves were selected to illustrate the limits of the observed variation between plants. Irregularities in the opening days of the experiment resulted from the large error involved in measuring small amounts of water transpired by the very young seedlings.

of the experiment, a decline which resulted both from the actual diminution in the rate of transpiration of diseased plants and from the increasing transpiring surface of the healthy plants. Our special interest lies less in this general decline than in any changes in rate of decline which may be coincident with late stages of the wilt disease.

Wilting was in progress in different individual plants from the thirteenth to the seventeenth day, and it is during just this period that the graph of the daily average transpirational ratio departs most distinctly from the otherwise nearly straight line. During this period, on the average, the diseased plants lost water more rapidly than was expected, only to show promptly a reduced rate of water loss after wilting was complete. This is shown more strikingly by the graphs drawn for plants W-9 and W-2. Both

these plants, during the several days of their wilting, showed a distinct increase in transpirational ratio, followed by a sharp decline after the leaves were all wilted. Clearly, in these two plants, wilting appears to have resulted not from a failure of the water supply but rather from a failure of water retention.

But not all 10 plants showed such a reaction. Plants W-10 and W-17, graphed in this same figure, are the exceptions. W-10 wilted rapidly on the fourteenth and fifteenth days, associated with a distinct reduction in the transpirational ratio such as would be expected if the wilting had resulted from water deficiency; and in W-17 the decline was gradual, graphing as a nearly straight line. Of the 10 diseased plants, 5 showed a distinct upward trend of the transpirational ratio coincident with wilting; 3 showed a questionable upward trend; and the 2 discussed here showed no such trend. The upward trend of the average curve is less decisive than inspection of the individual data suggests, due in part to failure of synchronization of the wilting in different plants.

In spite of their irregularity, however, these data seem to establish the fact that some plants, and probably most of them, suffer a rapid loss of water during wilting and that reduced availability of water, therefore, is not the chief factor, if even an important one, in the final wilting that characterizes the severe early development of this disease. Of more importance is some fundamental alteration of the protoplasts of leaf cells, probably their actual death, which leads to the loss of their normal powers of retaining water and consequently to their loss of turgor and to wilting of the leaf. That insufficient water supply may sometimes be involved is not disputed, particularly in the wilting of older plants. The rapid loss of water from collapsing leaves, indicated by this experiment, would subject the water supply of the plant to an extra strain and might lead, in plants with a reduced water-supplying capacity, to the wilting of other leaves from insufficient water. Such joint operation of these opposing factors might account for the behavior of plant W-17 indicated in figure 2.

SUMMARY

By following the transpirational histories of diseased and healthy plants the writer has obtained preliminary data which indicate that in the Fusarium wilt of peas wilting may result not from a diminished water supply but rather from an excessive loss of water from the leaves. This is thought to indicate the loss of the normal powers of water retention by the leaf protoplasts. It is sugested that this method of approach may yield significant facts regarding other vascular wilt diseases.

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STUDIES OF PATHOGENESIS AND RESISTANCE IN PEA WILT CAUSED BY FUSARIUM ORTHOCERAS VAR. PISI[1]

MAURICE B. LINFORD

INTRODUCTION

Although the wilt of peas (*Pisum sativum* L.) caused by *Fusarium orthoceras* App. and Wr. var. *pisi* Linford has been recognized only a few years, it is now known to be one of the major diseases of canning-crop peas in the United States.[2] The writer showed earlier (7) that this is characteristically a vascular disease, comparable in many respects with the well-known vascular fusarioses of cabbage, flax, tomato, and certain other plants, and that, in common with these diseases, it presents a number of interesting and fundamentally important biological problems arising from the specialized form of parasitism represented. In continuation of this earlier work the writer has now made both histological and experimental studies bearing upon the questions of pathogenesis and resistance. The histological findings are being published in a later paper which traces the interrelation of parasite and host during the infection of susceptible pea plants and which considers the histological manifestations of disease and of resistance. An earlier paper (8) has already dealt with rates of water loss during wilting. This present paper deals with experimental and observational studies bearing upon the questions of infection and factors which control it, the nature of changes in the host plant which constitute the disease, means whereby these changes are induced by the pathogene, and possible factors in resistance. The present status of knowledge concerning the diseases other than pea wilt which are dealt with here has been summarized so adequately in recent publications (3, 9, 11, 14) that the writer has attempted no general review of literature.

One monoconidial culture (No. 182c) of the pea-wilt fungus, isolated by the writer from a wilting pea plant at Madison, Wisconsin, in 1926, was used throughout all but the initial stages of this work. The Badger variety of pea was used chiefly as the susceptible host plant and Horal as the resistant pea, with others introduced for comparison when needed. The seed came chiefly from the upper Snake River Valley of Idaho.

[1] This paper is the second of a series reporting studies conducted in the Department of Botany, University of Wisconsin, under a fellowship grant from the National Research Council. The writer wishes to express his appreciation to Professor B. M. Duggar for his helpful advice throughout the progress of the work.

[2] Linford, M. B. Pea diseases in the United States in 1928. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. Suppl. 67. 1929.

PATHOGENICITY OF THE WILT FUNGUS

Although there was no reason to doubt the causal connection between the fungus and the disease under consideration, still certain unexplained variations in the natural and experimental development of pea wilt, which the writer had observed, suggested that some undetermined complicating factors might be involved. As a safeguard against misinterpretation of histological and other studies the following experiments were conducted to ascertain more definitely the pathogenic capacity of the pea-wilt Fusarium. Since it had already been shown that soil temperature is a major controlling factor in pathogenesis (7) these studies were conducted within the range of temperatures favoring the rapid development of disease.

PATHOGENESIS IN PURE AND MIXED CULTURE

To test the possibility that an association of other microorganisms with the wilt fungus might be essential for the development of this disease, inoculations were made under pure-culture conditions in large cotton-plugged culture tubes (3.5 x 20 cm. and 5 x 40 cm.). Water agar, soil-extract agar, and soil were used as substrata. Selected seeds from a clean lot of Badger variety were surface-sterilized[3] and, as a test of sterility, were planted an inch apart in Petri dishes of soft potato-dextrose agar. After germination had begun, the most uniform and vigorous seedlings were transferred individually with flamed forceps to separate culture tubes. These large tubes of soft agar (prepared with either tap water or soil extract with 1 gram of dextrose added per liter) were inoculated with the wilt fungus at the time the germinating seeds were planted. Roots and mycelium then penetrated the soft agar together to a depth of several centimeters and infection of the roots resulted. When sufficient substratum was provided and when the plants were allowed to stand long enough, leaf symptoms developed that, while different in detail from those which characterize the disease in open pot culture, were sufficiently similar to indicate their essential identity.

In early experiments, inoculation of tubes of sterile soil at the time germinating seeds were introduced led to the complete rotting of a high percentage of the seedlings. This difficulty was overcome by introducing the wilt fungus 1 week before the seedlings. Mycelium developed rapidly during this time and became clearly visible in the soil. Thereafter, mycelial growth was less conspicuous.

To compare the development of wilt in pure and mixed cultures, large

[3] The following procedure proved satisfactory: Wet the seed in 70 per cent alcohol, soak 1 minute in mercuric chloride (1–1000), rinse and soak in sterile water for 2 hours, treat a second time in mercuric chloride for 30 seconds, rinse several times in sterile water, and drain.

culture tubes of soil were prepared as indicated in table 1. Sterilization consisted of autoclaving the cotton-plugged tubes at 15-pounds pressure on 3 successive days. Soil with uniform moisture content was used, and approximately this content was maintained by the addition of sterile water from a volumetric pipette to restore loss as indicated by weight. Germinating peas were introduced 1 week after inoculation of the soil, and then 3 days later these tubes were stood in a flat of moist sand in the greenhouse in indirect sunlight where the air temperature varied between 18° and 26° C.

From table 1 it is seen that the wilt disease developed only in series E and F, inoculated with the wilt fungus after sterilization of the soil, with or without the supplementary addition of raw noninfested soil. Even the addition of 5 cc. of infested soil to the sterile soil in series D was without apparent effect, for the two plants in this series were still healthy after 40 days. In series E, inoculated with a pure culture of *Fusarium orthoceras* var. *pisi*, the disease developed rapidly and more typically than in the agar cultures. All 6 affected plants developed symptoms that were clearly characteristic of the disease, although not identical with those observed in open culture. Root injury was more pronounced than is typical of the disease and involved extensive cortical decay. Vascular invasion was relatively limited and, where it did occur, was less restricted to the xylem and more general in the cambium and phloem than is typical of the disease. These departures from the usual development are regarded as due solely to the peculiar environmental conditions within the cotton-plugged tubes, which modified both structure and function of the host plant.

From these results it is clear that the wilt fungus is capable of independent parasitism. Although it is not established that other organisms are never involved in a mutual relationship, the failure to detect any beneficial influence upon the disease of such mixed populations as were obtained in the experiment probably means that such a possible relationship is at least rare and unimportant.

ROOT WOUNDS IN RELATION TO INFECTION

The weak cortical parasitism of the pea-wilt fungus suggested that wounds might aid its entry into the vascular system. This was tested in two types of experiments. In several different plantings, young seedlings of susceptible and resistant varieties grown in flats of infested soil were subjected to root pruning. A long-blade knife thrust vertically into the soil was used to remove the young lateral roots from one or more sides of the taproot. In no case did such treatment lead to an earlier development of symptoms in susceptible plants or destroy the resistance of resistant plants.

TABLE 1.—*Development of Fusarium-wilt symptoms in Badger peas grown 25 days from partially germinated seeds in large culture tubes of soil prepared as indicated; a comparison of pure and mixed inoculation*

Series	Number of plants	Soil and its preparation	Condition of experimental plants 25 days after planting the partially germinated seeds
A	4	Thoroughly infested from former experiments.	Seedlings all decayed before germination was complete.
B	2	Sterilized.	Both healthy.
C	2	Sterilized, with 5 cc. raw soil added.	One healthy with roots browned slightly; one dwarfed and pale with roots partly decayed.
D	2	Sterilized, with 5 cc. infested soil added.	Both healthy.
E	8	Sterilized, with culture of wilt fungus added.	Wilt symptoms in six plants: growth of apical buds checked, stems swollen and succulent, leaves of two plants wilting and those of four pale and distorted. Two plants apparently sound.
F	4	Sterilized, with raw soil and wilt fungus added.	Wilt symptoms of unilateral dwarfing with leaflet distortion in one plant; one plant dying from decay of hypocotyl; two died early from seedling rot.
G	2	Sterilized, with cultures of wilt fungus and *Pythium* sp. added.	Both healthy. (Both died later from Pythium rot.)

In the second type of experiment, plants were grown 16 days, to the 5- or 6-node stage, in clean soil and then transplanted to clean and to infested soil. These seedlings were removed from the soil with care to minimize root breakage, and the controls were replanted directly. The plants to be wounded, however, were pruned severely: the taproot was cut back to 50 mm., lateral roots were cut to 10 mm., and, to provide a corresponding reduction of leaf surface, petioles were cut away, leaving only the stipules. Five plants each of Badger and of Horal peas were planted pruned and unpruned in clean and in infested soil, making 40 plants in all. In the clean soil plants of both varieties, whether pruned or not, recovered rapidly. In the unpruned controls even the tips of most lateral roots were unharmed, while in the pruned plants the roots branched out freely and new leaves were formed.

In the infested soil all plants of the susceptible Badger variety developed characteristic symptoms of the disease; and this development was distinctly earlier in the unpruned controls than in the pruned plants. Dead

and dying roots were more numerous on the plants that had not been pruned. The resistant variety, Horal, developed no wilt symptoms in aerial parts following either treatment, although some dead rootlets were found on both pruned and unpruned plants. Microscopic examination of many wounded roots failed to disclose any significant entry of the fungus into the vascular system through a wound. Wounding of fairly mature roots as carried out in these experiments thus appears to be unfavorable to the early development of this disease and to have no effect upon inherent resistance. This latter point is in agreement with the recently published work of Wade (13).

INFLUENCE OF AMOUNT OF INOCULUM IN THE SOIL

When peas are planted soon after an abundance of pure culture of the wilt fungus has been introduced into sterilized soil, even at a temperature favorable for the disease, pea wilt often develops slowly and irregularly. A delayed planting or a second planting after the first peas have been removed from the soil usually leads to an early development of the disease. A somewhat comparable phenomenon has been observed under field conditions in the natural development of pea wilt. The disease tends to develop earliest in parts of a field in which it has been present former years. Thus the areas that are infested one year will commonly show an earlier death of plants in the next planting than will the surrounding areas into which the wilt fungus has spread during the intervening time. Two of the possible interpretations of these phenomena are (1) a direct relationship between abundance of the fungus and promptness of infection and (2) a gradual and deleterious modification of the soil as a result of the presence of the fungus. To test the former of these possibilities the following experiment was conducted.

Peas were grown comparatively in thoroughly infested soil and in such soil diluted to various degrees. The infested soil had been inoculated after sterilization 10 months earlier and had since been proved highly infective. Dilution at the time of planting was in two parallel series, (A) with fresh uncooked soil and (B) with similar soil sterilized in the autoclave 6 days earlier. The infested soil was diluted to the fractional concentrations indicated in table 2, and each diluted sample was sifted repeatedly through a $\frac{1}{6}$-inch-mesh sieve to insure thorough mixing. Badger peas planted in 6-inch clay pots of this soil were thinned upon emergence to 10 seedlings, some of which were then lost from damping-off.

Table 2 presents the results of this experiment and figure 1 illustrates representative pots after 25 days. Control plants remained healthy throughout the experiment; all plants in the full-strength-infested soil were fully wilted in 25 days. In both dilution series the disease developed

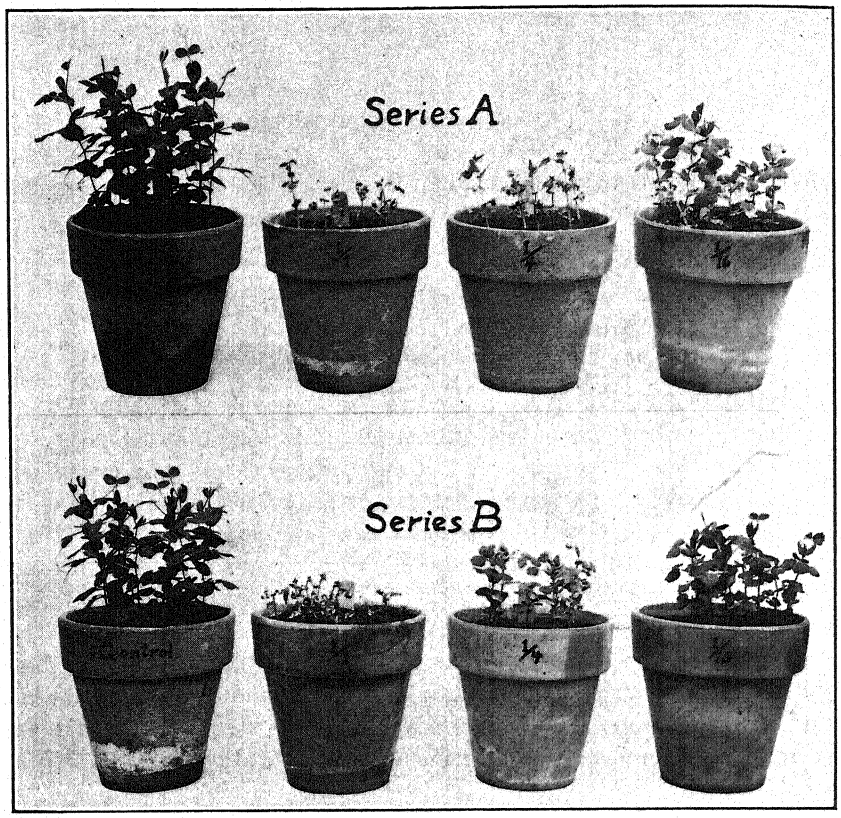


FIG. 1. Influence of concentration of inoculum upon the development of pea wilt. These photographs, taken 23 days after planting the seed, show progressive retardation of the disease with progressive dilution of the infested soil used as inoculum. Note that the control plants are larger in series A, diluted with uncooked field soil, and that the disease is more advanced in the various dilutions of this series than in series B, which was diluted with autoclaved soil. Compare with table 2.

more slowly as the degree of dilution increased; this was particularly conspicuous in series B, diluted with sterilized soil. Thus it is apparent that in plants exposed simultaneously to infection the disease may develop at very different rates, depending on the abundance of the wilt fungus in the soil or, at least, upon the proportion of infested soil in the soil mixture. This suggests that the rapid development of the disease in thoroughly infested soil may be a result of infection by the wilt fungus at many different points on the root system. The pronounced dwarfing which was observed in the greater dilutions (Fig. 1, B 1/16), even in the absence of other symptoms of the disease, suggests that in some way the wilt fungus may injure

TABLE 2.—*Influence of concentration of inoculum upon the development of Fusarium wilt of peas. Badger variety peas grown in the greenhouse where the temperature varied between 19° and 25° C.*

	Concentration of inoculum in soil at time of planting	Total plants	Days until first symptoms	Total plants affected after number of days indicated (per cent)					Plants wilted after number of days indicated (per cent)			
				17	19	22	25	27	19	22	25	27
Series A	Control	20	.....	0	0	0	0	0	0	0	0	0
Diluted with *fresh* field soil	1/1	6	15	100	100	100	100	100	0	100	100	100
	1/2	9	15	78	78	89	100	100	11	67	89	100
	1/4	7	15	57	86	86	86	86	0	14	71	71
	1/8	10	13	30	50	80	90	90	10	40	80	90
	1/16	9	15	33	56	67	89	89	11	22	44	67
	1/32	10	17	50	50	70	90	90	0	10	40	60
Series B	Control	19	.....	0	0	0	0	0	0	0	0	0
Diluted with *autoclaved* field soil	1/1	9	15	89	100	100	100	100	33	89	100	100
	1/2	5	15	40	60	100	100	100	0	0	60	80
	1/4	7	15	29	43	71	71	71	14	14	43	43
	1/8	2	27	0	0	0	0	50	0	0	0	0
	1/16	8	22	0	0	25	25	50	0	0	0	0
	1/32	5	22	0	0	20	20	40	0	0	0	0

the pea plant without entering the vascular system and thus completing infection.

MODIFICATION OF THE SOIL BY THE WILT FUNGUS

Some direct modification of the soil by the development of the wilt fungus might, if demonstrable, account for some of the variations indicated. Rosen (11) has suggested that the cotton-wilt fungus, *Fusarium vasinfectum,* which he reports has the property of reducing nitrates to nitrites, may cause nitrite injury to cotton roots and thereby produce the wilt disease without infection. His evidence seems uncertain at several points and Young, Ware, and Janssen (15) in later work found no support for the view. To test this with respect to pea wilt, several attempts were made to detect any possible toxic action of thoroughly infested soil which might aid in the development of early stages of the disease. Since only negative results were obtained, the work will not be described in detail. In one experiment, all water which reached the roots of plants grown in small porous cups passed in through the porous walls from an outer container of infested soil. In another, peas were grown in water culture in soil extracts from clean and from infested soil. In still another, infested soil was partially sterilized by various heat and formaldehyde treatments, but there was no development of symptoms in the absence of the fungus. Although these negative results are not wholly conclusive, they indicate very strongly that any influence upon the plant which the wilt fungus may have before it has become established in the vascular system of the roots is to be attributed to a direct action of the fungus developing upon the root surface or within the superficial layers of the cortex rather than to any indirect action through modification of the soil.

DISCUSSION OF PATHOGENICITY

These studies, although somewhat inconclusive at several points, suggest that the pathogenicity of the pea-wilt fungus is readily disturbed by various conditions in addition to the important controlling factor of soil temperature. The need for a well-standardized and ample inoculum in future studies of this disease is apparent, particularly in work that demands the use of sterilized soil, for it is clear that the rate of development of the disease may vary widely as a result of varied inoculation and that soil which has been sterilized with steam is relatively unfavorable for the disease. Such soil is likewise unfavorable for the healthy pea plant, which suggests again (7) that the disease develops best under conditions which, except for the presence of the wilt fungus, are most favorable for the growth of the healthy pea plant. The demonstration that the pea-wilt fungus is able to enter the vascular system and produce the disease without

the aid of other microorganisms and that such entry is not facilitated by gross wounds in the roots is in agreement with the earlier experience (7, pp. 34–36) that simultaneous infection with *Aphanomyces euteiches* Drechsler and the wilt fungus in no important manner affected the course of the wilt disease or lowered the degree of varietal resistance. These evidences, together with the narrow host range of the pea-wilt fungus, indicate that we are here dealing with a highly specialized type of parasitism.

It should be pointed out that recent advances in soil science, as recorded by Hendrickson and Veihmeyer (4, pp. 3–7), have shown the writer's earlier experimental work (7, pp. 14–17) on the influence of soil moisture on this disease to rest upon an unsound basis. The same is true generally of a great deal of phytopathological work in which essentially the same technique has been employed and the assumption made that different soil-moisture contents were being maintained experimentally.

ANALYSIS OF SYMPTOMS AS A KEY TO PATHOGENESIS

The writer pointed out earlier (7) that, in the Fusarium wilt of peas, wilting is not the most characteristic symptom and may, indeed, be completely absent when this disease assumes more the nature of cabbage yellows than of such wilt diseases as of tomato and flax. Whether death of the plant comes with wilting or with slow necrosis of the leaves, it is preceded by a series of conspicuous and diagnostically important changes which are significant indications of a disturbed physiology of the plant. Peas grown under conditions favoring early development of the disease exhibit the preliminary symptoms indicated in figure 2, A. Dwarfing results first chiefly from a reduced length of internodes and petioles but later, also, from retardation and then cessation of growth from the apical bud. Stipules and leaflets, reduced in size, become conspicuously rolled and distorted. The lower internodes of the stem increase in diameter and the entire shoot becomes more rigid, apparently from increased turgidity. The normal color gives way to slight yellowing of the leaves and to a superficial grayness suggesting an excess development of waxy bloom. Figure 2, B, illustrates the accompanying depression of root development.

Realizing that any attempt to understand the process of pathogenesis must have as a basis a clear picture of the disturbances in physiology and structure which constitute the disease, an attempt has been made to define more exactly the nature and extent of some of these changes.

For these studies, Badger variety peas were grown in deep flats of unsterilized clean soil and of infested soil that had been prepared by inoculation with the wilt fungus after sterilization several months before. They were grown in the greenhouse, where the temperature fluctuated between 18° and 25° C. With the few exceptions noted, diseased plants were se-



FIG. 2. A. Symptoms which precede wilting in the Fusarium wilt of peas. At the right is the upper portion of a healthy Badger pea seedling in the 7-node stage. At the left is a corresponding portion of a plant of the same age grown in wilt-infested soil and showing an extreme development of the characteristic symptoms which precede wilting in young plants. Note the shortened internodes and petioles, the dwarfed leaf laminae, the backward rolling of the lateral margins of the stipules, and the irregular distortion of the leaflets. Drawn from nature. B. Root dwarfing, a symptom of pea wilt. The root system at the left is typical of plants of the Badger variety grown 23 days from seed in the greenhouse in a 10-inch depth of clean soil. That at the right is of a plant of the same age and variety grown in wilt-infested soil. The shoot of this latter plant was wilting at the time this record was made, but there had been no appreciable root decay in either plant. Drawn from a photograph.

lected in which the preliminary symptoms were far advanced but before leaf necrosis or wilting had begun.

RATE OF DRYING OF HEALTHY AND DISEASED TISSUES

The writer observed earlier (7, p. 7) that diseased pea plants, when removed from the soil, wither more slowly than do healthy plants. This observation was tested quantitatively in the following comparison of rates of water loss from stem segments exposed to drying. Samples for this determination were each composed of 10 pieces of stem cut to the uniform length of 15 mm. Samples were prepared in duplicate from the aerial and subterranean portions of diseased and healthy plants, 28 days old from seed. In taking these samples, a block of stem, 5 mm. long, was cut away from the transition zone before the 15-mm. pieces were taken from the adjoining aerial and subterranean regions. Each sample was placed immediately in a dried and weighed weighing bottle with fitted stopper. With the aid of a

vernier caliper, measurements of stem diameter were made near the ends of every sample piece before the stems were cut.

Each bottle containing its sample was weighed and then its contents were spread to dry upon a fine wire screen, supported 14 inches above the table top, in a dark room where the temperature remained within the limits of 26.6° and 27.7° C. After 2 hours exposure the samples were returned to their bottles and weighed, after which they were again spread to dry. Alternate drying and weighing then proceeded until all samples had come to constant weight, the periods of drying being lengthened as the rate of water loss declined. Figure 3 presents graphically the results of this determination, each curve representing the average of duplicate samples which checked closely throughout.

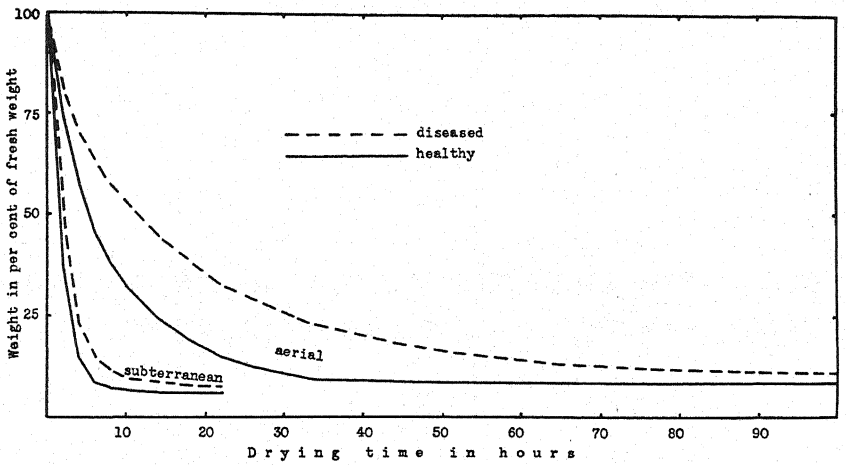


FIG. 3. Comparison of rates of drying of aerial and subterranean stem samples of diseased and healthy pea plants when exposed to the dry air of a constant-temperature dark room. Each curve represents the average of duplicate samples of 10 pieces of stem each, prepared as described in the text.

The healthy stem samples, both aerial and subterranean, lost water much more rapidly than the diseased and came to equilibrium with the air of the drying room in approximately half the time. The greatest contrast, however, came in the rapid drying of subterranean as compared with aerial samples, both healthy and diseased. This suggested that part of the difference between the healthy and diseased might lie in a different condition of the epidermal covering. A less detailed comparison was therefore made in which the sample pieces were split lengthwise before exposure. These lost water much more rapidly than did the unsplit, but still a striking difference between healthy and diseased pieces indicated that at least part of the water retentiveness results from a direct change in the internal tissues

of the stem. Comparative determinations indicated that leafy shoots dried to equilibrium less rapidly than the stem samples but showed a similarly great difference between diseased and healthy plants.

DRY MATTER AND ASH CONTENT

After these samples had reached equilibrium with the air of the drying room, they were dried to constant weight in the oven at 110° C. They were then transferred to baked-porcelain crucibles and incinerated to constant weight in an electric furnace. All the data are presented in table 3.

Striking differences between diseased and healthy plants are shown by these data with respect to the several points compared. In cross-sectional area the diseased aerial stems were 22.7 per cent greater than the healthy; in weight they were 44.5 per cent greater; and in computed specific gravity they were 13.5 per cent greater than the healthy. Consistently, the diseased plant samples showed a larger percentage of dry matter than did the healthy, the difference being greater in the subterranean samples (52.7 per cent) than in the aerial (25.9 per cent). Ash was almost directly proportional to dry matter, amounting to about 11.0 per cent of the oven-dry weight in all samples. It was thus more abundant on a fresh-weight basis in the diseased plants. The diseased stems not only contained a smaller percentage of water in the fresh condition but they retained more of it in the air-dry condition than did the healthy stems. Hygroscopic water amounted to 6.1 per cent of the dry weight in the healthy aerial stems compared with 8.7 per cent in the corresponding diseased stems.

OSMOTIC VALUE OF DISEASED AND HEALTHY TISSUES

The above data as well as evidence from histological studies suggest that important changes in cell contents throughout the plant are involved in the development of this disease. To test this further the plasmolytic method was employed for a comparison of the osmotic value of cell sap in healthy and diseased stems. The plants used were grown as indicated above but were in later stages of the disease and showed abscission or wilting of parts of some but not of all leaves. For comparison a few plants were taken in other stages of the disease. The tissue employed was the outer one or two layers of green subepidermal parenchyma from the second internode of the stem. This was chosen because of the ease with which suitable plastid-bearing cells were obtained from a uniform position by stripping off a portion of the epidermis with bits of adherent cortical tissue. Test solutions were prepared by dilution with double-distilled water from a molar solution of sucrose, and the actual tests were made in hollow-ground microscope slides. A separate plant was used for each of

TABLE 3.—*Comparison of stem size and of dry matter and ash contents of healthy Badger variety pea plants and of plants affected with the Fusarium wilt. All figures for weights, in milligrams, are average readings of duplicate samples of each of 10 pieces of stem. Figures for area and volume, in square millimeters and cubic millimeters, respectively, are averages based upon the single piece of stem as the unit*

	Samples of aerial stem				Samples of subterranean stem			
	Healthy	Diseased	Difference		Healthy	Diseased	Difference	
			Amount	Per cent			Amount	Per cent
Area of epidermis	111.0	123.6	12.6	11.3	114.0	121.6	7.6	6.7
Area of two cut ends	8.8	10.8	2.0	22.7	9.2	10.5	1.3	14.1
Volume	65.7	83.8	18.1	27.5	69.1	86.3	17.2	24.9
Computed specific gravity	0.96	1.09	0.13	13.5	0.83	0.87	0.04	4.8
Weight of fresh sample	631.0	912.0	281.0	44.5	575.0	748.0	173.0	30.1
Air-dry weight	54.0	101.0	47.0	87.4	34.0	66.0	32.0	94.1
Percentage of fresh weight	8.6	11.1	2.5	29.1	6.0	8.8	2.8	46.7
Oven-dry weight	51.0	93.0	42.0	82.4	31.9	60.5	28.6	89.7
Percentage of fresh weight	8.1	10.1	2.1	25.9	5.5	8.4	2.9	52.7
Hygroscopic water, per cent	6.1	8.7	2.6	42.6	7.1	8.7	1.6	22.6
Weight of ash	5.7	10.1	4.4	77.2	3.7	6.6	2.9	78.4
Percentage of oven-dry weight	11.0	10.6	0.4	3.8	10.8	10.7	0.1	0.9
Percentage of fresh weight	0.91	1.10	0.19	20.9	0.64	0.88	0.24	37.5

the 70 test mounts examined in this study. The average figures for osmotic values thus determined are set forth below, together with the concentration percentages of the isosmotic sucrose solutions and the calculated equivalent osmotic pressures:

	Healthy	Diseased
Osmotic value at incipient plasmolysis in terms of molar sucrose solution	0.37	0.54
Percentage concentration of sucrose in the isosmotic solution	12.7	18.5
Osmotic pressure, in atmospheres, of isosmotic sucrose solution at 22° C.	8.96	13.08

These average figures indicate an osmotic value 46 per cent higher in the diseased than the healthy plants. Of the healthy individuals tested none showed plasmolysis in 0.3 molar sucrose, while all plasmolyzed in 0.38. A few young plants gave plasmolysis in 0.35 and 0.36 molar solutions. Of the diseased plants, none gave plasmolysis in 0.53, but all did in 0.60 molar and stronger solutions. Practically all affected plants in medium and medium-late stages of the disease reacted similarly, but plants which showed very early symptoms and those that already had withered back to the succulent stem base commonly gave plasmolysis in solutions somewhat less concentrated than 0.53 molar. No evidence of extreme permeability of the cell membranes was detected in these determinations. Cells remained plasmolyzed without apparent change in the sugar solutions for 10 minutes or longer but recovered promptly when the solution was diluted or replaced with water.

REGENERATION OF AFFECTED PLANTS

When conditions prevent the rapid development of the disease, dying plants commonly produce one or more lateral shoots from their basal nodes. As shown in figure 4, such shoots may develop after the primary stem is almost dead and may remain healthy after it is completely withered. A number of sprouts may arise successively and grow to a height of several centimeters before each in its turn collapses. In the rapid development of the disease such sprouting may be absent or may be reduced to the formation of a few short stems which soon wither.

Regenerative ability is shown also by the experiment summarized in table 4 on the rooting of cut stems in water. Perfection variety peas, 26 days old from seed, some healthy from clean soil and others in early and medium stages of the disease, were washed from the soil, cut under water just above the cotyledons, and placed in culture tubes of tap water. These were held 8 days in the greenhouse before the data were taken. All the

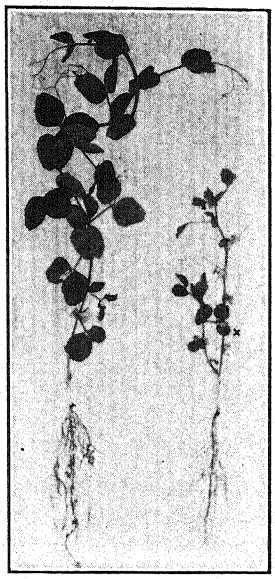


FIG. 4. Sprout from the basal internodes of a pea plant affected with the Fusarium wilt disease. The diseased plant on the right has undergone progressive withering of leaves up to the apical bud without a distinct wilt phase and has sprouted from the second node, as is characteristic of the slow development of this disease. The cross (x) represents the height to which the fungus extended in the primary shoot as determined microscopically. This sprout showed no symptoms of the disease. Horsford's Market Garden variety peas grown 47 days in sparingly inoculated soil in a cool greenhouse where the temperature fluctuated between 14° and 20° C.

TABLE 4.—*Production of adventitious roots and axillary shoots by healthy and diseased pea stems in a period of 8 days*

Plant number	Stems from healthy plants				Stems from diseased plants			
	Adventitious roots		Axillary shoots		Adventitious roots		Axillary shoots	
	Number	Total length	Number	Total length	Number	Total length	Number	Total length
		Mm.		Mm.		Mm.		Mm.
1	3	72	0	0	10	367	0	0
2	3	36	2	12	12	330	0	0
3	4	36	0	0	9	128	1	23
4	0	0	0	0	13	201	1	37
5	2	3	0	0	3	35	1	22
6	3	72	0	0	9	95	1	12
7	2	4	0	0	...	.....	...	.....
Average	2.4	31.9	0.3	1.7	9.3	192.7	0.7	15.7

diseased plants, with the exception of number 5, which, initially, was in a more advanced stage of the disease, developed a larger number of roots and a greater combined root length than any of the healthy plants. Shoots appeared on 4 of the 6 diseased and on only 1 of the 7 healthy plants. Moreover, in 4 of the diseased plants, growth of the apical bud was resumed with the formation of new leaves free from rolling and distortion.

DISCUSSION OF SYMPTOM STUDIES

These various data and observations indicate clearly that the wilting or death of an affected pea plant is preceded by a series of profound disturbances in metabolism and growth which cannot well be ignored in future studies of this and related diseases. Theories of pathogenesis that have been focused chiefly upon the process of wilting and that have stressed the possible diminution of water supply to the leaves appear completely inadequate, for the actual wilting occurs, in this disease at least, as a relatively unimportant culmination of a long series of changes that are in no way simulated by experimentally-induced drouth. It seems probable that both the hypertrophy of parenchymatous cells and the increased rigidly of affected plants are attributable to the increased osmotic value of the cell contents which results, in turn, from accumulations of soluble reserves. In part, however, the rigidity is due to an increased thickness of cell wall and an abnormally early maturation of the skeletal tissues of the shoot. The marked increase of organic as well as inorganic reserves in diseased plants, together with the regenerative ability of such plants, suggests that the symptoms considered here do not result from a general starvation of the plant as a whole. Either the synthesis of abnormal materials poorly suited to the growth needs of the plant or an interference with the utilization of normal synthetic materials for growth is suggested. Failure of translocation is indicated particularly by the sprouting of affected plants from their basal nodes after apical growth of the primary axis has stopped.

The suspicion that a carbohydrate-high condition was indicated was not justified when graded applications of calcium nitrate were without apparent effect until applications were used that dwarfed even the check plants in noninfested soil. It is suggested that chemical analyses of the reserves accumulated in diseased plants might yield a more definite clue to the nature of the basic physiological disturbances involved.

WILTING INDUCED BY TOXIC CULTURE FILTRATES

Following the demonstration by Hutchinson (6), Brandes (1), and others, that a liquid culture medium in which a pathogenic microorganism has grown will cause wilting when introduced into cut stems of the host

plant, considerable attention has been devoted to toxicity theories of the pathogenesis of those wilt diseases in which the pathogene invades primarily the vascular tissues. Assuming that a fungus liberates the same toxic substances within the vascular system of its host plant as it does in a staling culture, a study of the wilting induced experimentally in cut stems by culture filtrates or fractions of such filtrates should provide a key to the nature of wilting and of the toxic substances involved in pathogenesis.

Haymaker (3) has presented evidence recently in support of the view that such studies are applicable to the problem of resistance as well as of pathogenesis. Working with the Fusarium wilt of the tomato he was able to simulate the symptoms of the disease more closely than others have done, including some aspects beyond mere wilting and collapse of leaves and stems. Moreover, he found a direct correlation between degrees of pathogenicity of *Fusarium lycopersici* and toxicity of its metabolic products. Culture filtrates were more toxic when the fungus was grown at the optimal temperature for pathogenesis than when grown at other temperatures. Filtrates from cultures of highly pathogenic strains of the fungus were more toxic than those from weakly pathogenic strains. And, of special interest in this present consideration, he demonstrated with many varieties of tomato what White (14) had found with two, that varieties, resistant to the wilt disease, are less readily damaged by these culture filtrates than are the susceptible varieties.

EXPERIMENTAL STUDIES

To determine the applicability of these findings to the problems of pathogenesis and resistance in pea wilt, the writer has used chiefly the technique of placing cut pea stems into filtrates from solution cultures of the wilt fungus and observing the wilting or other injury that resulted. Pea seedlings were grown in 12-inch clay saucers of autoclaved soil in the greenhouse where the temperature varied between 19° and 25° C. All saucers were kept under the same conditions on the same bench. Seedlings were used at the age of 20 to 23 days in the different experiments. They were washed from the soil to avoid breaking; the roots were washed clean; and each stem was cut off under water with a razor blade at a point just above the cotyledonary node. The cut stems were then rinsed in boiled water and transferred to the test solutions.

For the toxic culture filtrates, strain 182c of *Fusarium orthoceras* var. *pisi* was grown in White's modification (14) of Richards' Solution,[4] with 250 cc. in each liter flask. Cultures were incubated 15 to 22 days in the different series at room temperature, 20° to 24° C. The filtrate was then

[4] KNO_3, 10 gm.; KH_2PO_4, 5 gm.; $MgSO_4$, 2.5 gm.; $FeCl_3$, 20 mg.; dextrose, 50 gm.; and water, 1,000 cc.

TABLE 5.—*Varietal susceptibility to the toxic action of filtrates from Richards' solution cultures of the pea-wilt fungus, Fusarium orthoceras var. pisi, and of solutions of the alcoholic precipitate from such filtrates*

Varieties susceptible (S)[c] or resistant (R)[c]	Index of wilting[a] after 24 hours in the indicated dilutions of the following solutions											Index of flagging[b] after 19 hours in the indicated molar solutions of dextrose			
	Filtrate from Richards' Solution cultures of the pea-wilt fungus								Solution of alcoholic precipitate from filtrate						
	Trial I				Trial II										
	F/1	F/2	F/4	Total	F/2	F/4	F/8	Total	APS/1	APS/2	Total	0.8	0.6	0.4	Total
Badger (S)	10	7	4	21	6	3	0	9	2	0	2	9	0	0	9
Horsford "	9	7	5	21	5	3	1	9	4	0	4	9	6	0	15
Advancer "	10	8	8	26	7	4	2	13	10	1[d]	11[d]	15	11	0	26
Perfection "	10	4	3	17	6	4	2	12	2	0	2	9	9	0	18
Horal (R)	10	9	6	25	7	5	1	13	3	1[d]	4[d]	3	0	0	3
Rogers' K "	10	8	6	24	7	5	2	14	4	0	4	15	15	11	41
Green Admiral "	6	6	4	16	5	2	0	7	2	0	2	15	15	0	30
White Admiral "	10	9	5	24	5	4	2	11	5	0	5	15	15	6	36

[a] Index of wilting based upon the following values: Leaves and upper third of stem fully withered = 5; leaves withered but stem scarcely affected = 4; all leaves withered but bud and petioles scarcely affected = 3; some but not all leaves fully wilted or withered = 2; and some leaves wilting but none fully withered = 1.

[b] Index of flagging based on the following values: Stem fully shriveled at base and leaves far wilted = 5; and plant flaccid but not shriveled = 3.

[c] As determined by field and greenhouse tests in which the seed is planted in infested soil. Resistance and susceptibility are sharply defined under these conditions.

[d] One plant was lost during the experiment, otherwise this figure might have been greater.

prepared by repeated passage through fine filter paper and was diluted as needed by the addition of boiled distilled water. Controls consisted of boiled water and of sterile Richards' Solution, filtered and diluted as indicated.

Preliminary experiments with this technique indicated that both resistant and susceptible varieties of peas will wilt when treated in this way. A more extensive experiment was then conducted, as indicated in table 5, trial I, to compare in detail the responses of resistant and susceptible varieties. Seedlings of 8 varieties, 21 days old, were prepared as indicated above and placed singly in duplicate culture tubes of water, of sterile Richards' Solution, and of filtrate from 15-day cultures, the latter two in three concentrations; full-strength, half-strength, and quarter-strength. All plants in water and in the diluted Richards' Solution (Fig. 5, plant 5) remained fresh and turgid. In the full-strength Richards' Solution a general flagging (Fig. 5, plant 4), very different from wilting, was observed in all varieties except Green Admiral (R)[5] and Perfection (S),[5] which

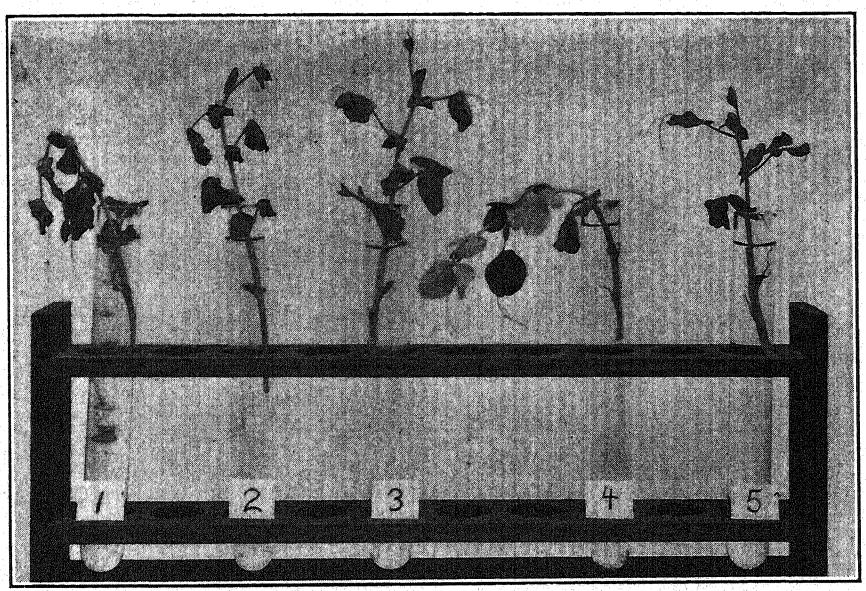


FIG. 5. Wilting of the disease-susceptible Horsford variety in stale culture filtrates and flagging in Richards' Solution. These excised stems were photographed after 19 hours in solutions as follows: 1, full-strength culture-solution filtrate; 2, half-strength filtrate; 3, quarter-strength filtrate; 4, full-strength sterile Richards' Solution; and 5, quarter-strength Richards' Solution. Note the distinction between wilting, as shown by plants 1, 2 and 3, and flagging, as shown by plant 4. See trial I, table 5.

[5] (R) and (S) represent resistant and susceptible varieties, respectively. See footnote [c], table 5.

remained turgid. Wilting occurred in the culture filtrates in all varieties in proportion to the concentration of filtrate. Furthermore, the 8 varieties differed considerably among themselves in their susceptibility to injury, as is seen by inspection of the column of totals under trial I. While the highest index of wilting[6] observed, 26, was for the susceptible variety Advancer, and the lowest, 16, for the resistant Green Admiral, the total of the four indices for the disease-resistant varieties was slightly greater than for the susceptible varieties. In other words, while there were distinct differences in the reaction of the several varieties, there was no apparent relationship between this and resistance or susceptibility to the wilt disease.

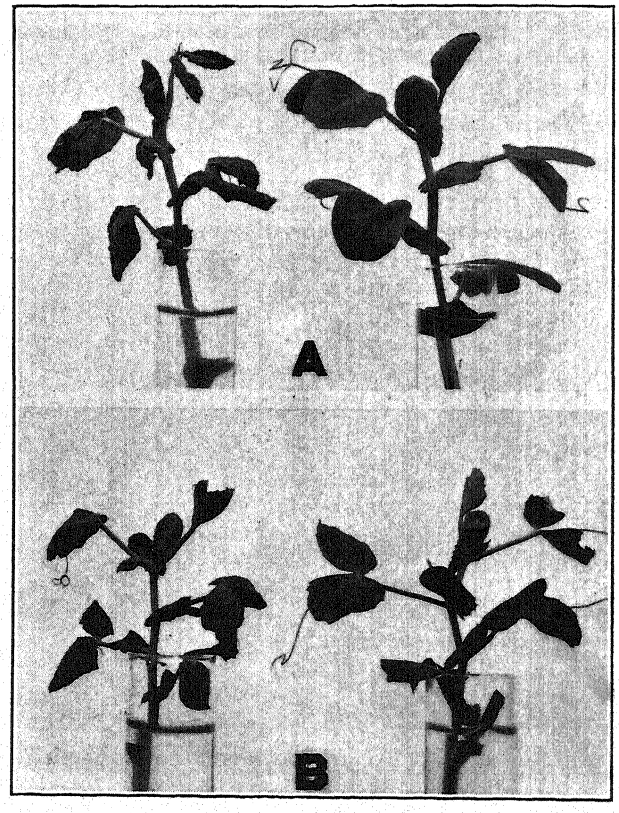


FIG. 6. Pea shoots wilting in stale culture filtrates after 20-hours exposure. See trial II, table 5. A. Type of injury observed in the disease-susceptible variety, Advancer. The plant at the left has stood in half-strength filtrate from a 22-day culture of the pea-wilt fungus in Richards' Solution; that at the right in half-strength sterile Richards' Solution. B. Comparison of wilting in the disease-resistant variety Horal (left) and the disease-susceptible Badger (right) in half-strength filtrate from a 22-day culture.

[6] See footnote [a], table 5.

In another experiment, trial II of table 5, 20-day seedlings of the same 8 varieties were tested with filtrates from 22-day cultures. To avoid flagging and to retard wilting, full-strength filtrates and Richards' Solution were omitted and $\frac{1}{8}$-strength solutions were added. Figures 6, 7 and 8 illustrate some of the test plants after 20 hours. In water and in the diluted Richards' Solution, all plants of all varieties remained turgid after 24 hours. In all dilutions of filtrate, all showed some injury with the exception of 2 varieties in the greatest dilution. Wilting, again in this experiment, was proportional to the concentration of the filtrate and varied from one variety to another but was not related to the resistance or susceptibility of these varieties to the wilt disease. For example, figure 6, B shows distinctly greater injury in the Horal (R) than the Badger (S), and figure 7, comparing 2 resistant varieties, shows much greater injury in Rogers' K than in Green Admiral. In this trial the total index of wilting was again slightly greater for the 4 resistant than the 4 susceptible varieties.

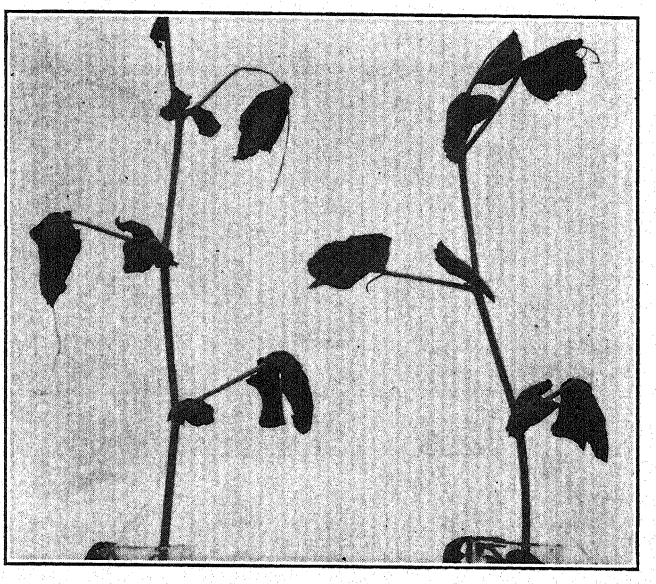


FIG. 7. Comparison of wilting in two disease-resistant varieties in stale culture filtrates. These plants, Rogers' K at the left and Green Admiral at the right, have stood 20 hours in half-strength filtrate from a 22-day culture. See trial II, table 5.

Since this observed absence of correlation between resistance to disease and resistance to toxic wilting was directly opposed to the findings of Haymaker (3) for tomato, another test was made using only the alcoholic precipitate fraction instead of the entire filtrate. Clear filtrates from 20-day cultures were precipitated with 2 volumes of 95 per cent alcohol,

TABLE 6.—*Eight varieties of peas listed in the descending order of their susceptibility to wilting under the conditions indicated, their susceptibility to flagging in dextrose solutions, and the relative heights of their shoots at the time of testing. Two or more varieties within brackets are of equal rank. Compare with table 5*

Susceptibility (S) and resistance (R) to Fusarium wilt		Susceptibility to wilting from toxic action			Susceptibility to flagging in graded solutions of dextrose	Relative heights of varieties at time of testing
		Filtrate from culture of wilt fungus in Richards' Solution		Aqueous solution of alcoholic precipitate		
		Trial I	Trial II			
(S)	Advancer	Advancer (S)	Rogers' K	Advancer	Rogers' K	Rogers' K
	Perfection	Horal (R)		White	White	White
	Badger		Advancer	Admiral	Admiral	Admiral
	Horsford	Rogers' K (R)	Horal		Green	Green
		White		Horal	Admiral	Admiral
		Admiral (R)	Perfection	Rogers' K	Advancer	Advancer
			White	Horsford		
		Badger (S)	Admiral		Perfection	Horsford
		Horsford (S)		Badger		
			Badger	Perfection	Horsford	Badger
(R)	Horal	Perfection (S)	Horsford	Green		
	Green			Admiral	Badger	Perfection
	Admiral	Green	Green			
	White	Admiral (R)	Admiral		Horal	Horal
	Admiral					
	Rogers' K					

washed with alcohol, dried, and then digested 2 hours at room temperature with $\frac{2}{3}$ the original filtrate volume of distilled water. The solution was then filtered and diluted for use and was tested in duplicate with 23-day plants of all 8 varieties. This solution proved less toxic than the whole filtrate and produced no visible injury in dilutions greater than half-strength in the 24-hour period. Even at the full strength the injury produced was relatively slight, as shown in table 6, and yet again there were distinct differences in varietal behavior that were not correlated with the susceptibility of these varieties to the Fusarium wilt.

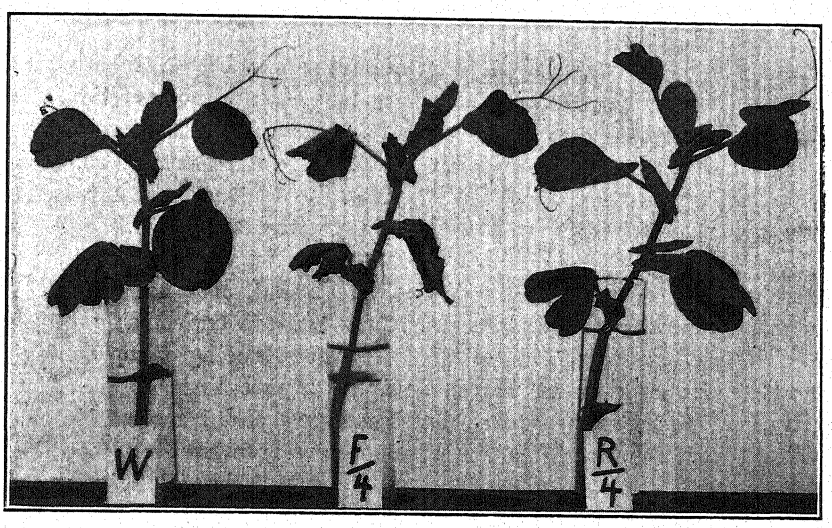


FIG. 8. Wilting of the disease-susceptible variety Advancer in stale culture filtrate. These plants have stood 20 hours in (left) distilled water, (center) quarter-strength filtrate from a 22-day culture, and (right) quarter-strength Richards' Solution. Note that the water lost from these tubes, chiefly through transpiration, is much greater in the case of the culture filtrate, where wilting occurred, than in the Richards' Solution, where all leaves remained turgid.

In trial I the 2 varieties least damaged by the filtrates, Green Admiral and Perfection, were the only 2 that did not flag in the full-strength Richards' Solution. This suggested that some difference in osmotic values might account for the observed varietal differences in susceptibility to injury. Accordingly, after a preliminary trial to determine suitable concentrations, cut stems of 15-day plants were placed in triplicate in the following solutions of dextrose: 0.4, 0.6, and 0.8 molar. After 19 hours the index of flagging was computed as indicated in footnote ([b]), table 5, as a numerical basis for comparison of the varieties. The varietal differences thus revealed are much greater than in the toxicity tests but show no apparent correlation with either toxic injury or the wilt disease.

For more ready comparison the results of these several tests have been summarized in table 6, where the 8 varieties are listed in the descending order of their susceptibility to injury in each of these tests and in the descending order of stem length at the time of testing. Comparison of these listings reveals a general agreement between trials I and II and the alcoholic precipitate test, in spite of disagreement in detail, but shows a complete lack of agreement between these listings and the resistance or susceptibility of these varieties to the wilt disease. In trial I, 3 of the 4 most damaged varieties were resistant to the disease; in trial II, 2 of the first 4 in the list are resistant; and, in the alcoholic-precipitate test, 3 of the first 5 are resistant.

Susceptibility to flagging in dextrose solutions throws these varieties into an entirely different order of rating, which shows no correlation with either resistance to the disease or to toxic action but which correlates very closely with the heights[7] of the plants at the time of testing. From this it appears likely that the collapse observed in the dextrose solutions is not a direct measure of the relative osmotic values of the test plants.

Injury in the toxic filtrates was not proportional to height of plant. Rogers' K, one of the tallest varieties, and Horal, only half as tall, are among the most susceptible to toxic injury and are both highly resistant to the disease. As a further test of the possible bearing of morphological characters, an experiment was made with 2 pure lines of the Alcross variety,[8] identical in appearance, but 1 resistant and 1 susceptible to the disease. Two 19-day plants of each pure line were tested for susceptibility to injury in 4 different concentrations of: (a) Richards' Solution; (b) filtrate from 17-day cultures filtered through paper alone; (c) filtrate from similar cultures filtered through paper and alundum cups; and (d) aqueous solutions of alcoholic precipitate from the paper filtrate (b). Observations over a period of 90 hours revealed no consistent differences between these strains of peas, in spite of the sharply drawn distinction in their reaction to the disease.

In a minor supplementary test, toxic culture filtrates were introduced through cut petioles into the stems of growing plants without disturbing the roots. After 1 week the plants thus treated showed various external symptoms, none wholly characteristic of the disease. Microscopic examination revealed no great disturbance in control plants where water alone was used, but, in both Horal (R) and Horsford (S), the introduction of culture filtrates had led to a limited amount of gumming of the vascular tis-

[7] Differences in height are the result chiefly of differences in length of stem internodes; in fact, 2 of the shortest of these varieties had slightly more nodes at a given age than 4 of the varieties of intermediate height.

[8] This seed was kindly furnished by Mr. Earl J. Renard.

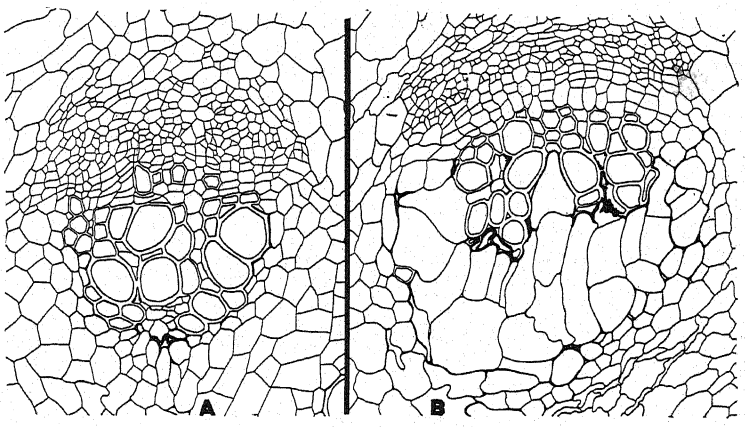


FIG. 9. Hypertrophy of xylem and adjacent parenchyma of a leaf trace in response to the introduction of filtrate from a 15-day Richards' Solution culture of the pea-wilt fungus into a Horsford pea plant through a cut petiole. Drawn with the aid of a camera lucida, ×173. A. Normal leaf trace in the fourth internode, a few millimeters below the cut petiole and on the opposite side of the stem. B. The affected leaf trace, drawn from the same section as A. Note the extreme hypertrophy of parenchyma between and around the xylem vessels; the more moderate hypertrophy of the cambium and metaphloem region; the collapse of some of the protoxylem; and the crushing of thin-wall pith parenchyma.

sues and to a conspicuous hypertrophy of xylem and surrounding parenchyma in and around the stipule traces and leaf traces involved (Fig. 9). The presence of pieces of mycelium in these leaf traces makes it impossible to attribute all of this disturbance to the toxic substances introduced; yet the promptness with which the hypertrophy occurred in proportion to the small amounts of living fungus present suggests that the response was chiefly to the filtrate itself. No check with sterile Richards' Solution was used. The similarity of this to the hypertrophy of cambium and stelar parenchyma in the development of the pea-wilt disease (7) suggests that this type of study merits further application in the testing of toxic culture filtrates.

DISCUSSION OF TOXICITY

From these studies it is evident that resistance to the Fusarium wilt of peas is not a question of resistance to staling products of the wilt fungus. This is directly contrary to the finding of Haymaker (3) with respect to resistance to the Fusarium wilt of tomato.

The type of injury produced by toxic culture filtrates did not simulate closely the symptoms of pea wilt, but this was not expected. Symptoms of this disease require for their development a period of several days during

which a small but increasing effect of the fungus must be operative, and the conditions imposed by this technique made this impossible. The wilting observed in these experiments did, however, appear as closely comparable to the collapse of diseased plants as might be expected in the absence of the preliminary changes in the plant typical of the disease.

In spite of the criticisms of Hursh (5) and others, this general technique appears to be valid, and extremely useful in studies of some aspects of pathogenesis. Clearly, this wilting was a result or accompaniment of collapse of the cells of, first, the leaves and, then, the stems under the influence of toxic substances in the filtrates and not a consequence of a diminished supply of water to the leaves. Unlike the conditions described by Haymaker (3) for the tomato and illustrated by Hursh (5), this toxic injury did not first involve a general flaccidity of the succulent parts of the plant except when the strongest solutions were employed. With the more dilute solutions the affected leaflets or their margins first became water-soaked in appearance and dark green in color, then lost their turgor, withered, and dried. Collapse of petioles and stem proceeded only after leaflet collapse, as shown by figures 6, 7 and 8, a very different sequence from that observed in the flagging of plants from lack of sufficient water in strong Richards' Solution (Fig. 5, plant 4) or in the dextrose solutions.

As appears from transpiration records (8) to be the case in the wilting of diseased plants, wilting of these cut stems was accompanied by rapid loss of water. This question arose after the experimental work was concluded, but examination of the writer's extensive photographic records of the experimental plants showed repeatedly, as indicated in figure 8, that more water was lost during the experimental period by the wilting plants than by healthy plants in the corresponding concentrations of Richards' Solution. The osmotic value of sterile Richards' Solution was probably nearly the same as that of the toxic filtrate. White (14, p. 229) found no significant difference in a similar case. It is clear, then, that the wilting plants in toxic solutions transpired or lost by evaporation from necrotic surfaces considerable amounts of water. The toxic substances were acting directly upon the leaf cells and not, as suggested by Hursh, primarily damaging the vascular system of the stem. This is indicated further by the fact that Horal and Rogers' K varieties, one only half as tall as the other, reacted in the same way in spite of different lengths of vascular system, the reaction being primarily in the leaves. This technique has not simulated the late wilting of large plants, which may sometimes involve a diminished supply of water to the leaves.

GENERAL DISCUSSION

The course of development of the pea wilt disease is more varied than might perhaps be implied from the foregoing considerations. The dis-

ease results from the interaction of one pathogenic fungus with different varieties of the host-plant species under diverse conditions, as of nutrition, temperature, and moisture; and both the course and end point of the interaction vary with the conditions. The course of the disease set forth here is typical for conditions which favor the rapid development of the disease in young plants. When, however, conditions are less favorable or when the host is approaching maturity at the time symptoms first appear, the sequence of events is different. Throughout this variation wilting is not the most constant symptom, for it may be completely displaced by a slowly progressing but none the less complete collapse. This suggests therefore that wilting merits proportionately less attention than that given it by some investigators and that the causes of necrosis, whether slow or rapid, be given more consideration; particularly, since in this disease the characteristic wilting appears to differ from slow necrosis, chiefly in rate of progress.

Wilting in older plants may be a different phenomenon. In pea plants that are half grown or larger when the disease develops, symptoms are more nearly those of the conventional wilt disease than those described in this paper. Preliminary symptoms may be much reduced; particularly, in plants that have attained most of their stem growth, but they can be detected even in nearly mature plants. It is in such large plants that indications of alternate wilting and recovery have been noted. Necrosis is sometimes less intimately associated with this late wilting; and thus there is the suggestion that a diminished supply of water may stand in a causative relationship, particularly, since microscopic examination of the taproot and stem base of such plants commonly reveals a great abundance of the fungus in the xylem vessels. While this mycelium, in itself, probably does not greatly hinder the passage of water, still, through the liberation of carbon-dioxide as suggested for *Fusarium lini* by Tochinai (12), it might effectively obstruct the passages. However, even in the older plants, symptoms which precede wilting indicate a gradual disturbance of synthetic or assimilative processes of the host; and the close association of necrosis with wilting sometimes observed in such older plants suggests that along with a diminishing supply of water there is progressively increasing yield of toxic metabolic products being carried up to the leaves from the increasing quantity of mycelium, and that such toxic substances are operative along with shortage of water in causing the collapse.

In younger plants, wilting and necrosis appear to be the culmination of a series of important changes in metabolism and growth of the host brought about by the fungus through the action, apparently, of products of its own metabolism. The possibility of toxic products being liberated by damaged host cells, as pointed out by Overton (10) and Haskell (2), must

be admitted. That such collapse is not a result of general starvation is shown by the accumulation of organic and mineral reserves and by the regenerative ability of affected plants. This does not prove, however, that local starvation, through failure of translocation, or unbalanced starvation through unfavorable utilization or synthesis, may not be significant. Moreover, as shown in a foregoing paper (8), water is lost at an apparently increased rate during rapid collapse of young plants. This further supports the view that the total injury is a result not of lack of water but rather of a complex and systemic toxic action which causes death of the leaves and stem only after a long series of disturbances in the metabolic and growth balance of the plant.

Resistance, inherent in some varieties of peas, appears to lie either in obstruction of entry of the pathogene into the vascular system of the pea plant or in resistance to its growth after such entry. Contrary to the expectations raised by the work of White (14) and of Haymaker (3), resistant varieties of peas, as a group, are no more tolerant of toxic culture filtrates than are the susceptible varieties. Other aspects of resistance are considered in following papers.

SUMMARY

This paper is one of a series reporting studies of pathogenesis and resistance in the wilt of peas caused by *Fusarium orthoceras* App. and Wr. var. *pisi* Linford carried out as an incumbent of a National Research Council Fellowship in the Biological Sciences. Histological investigations are being reported separately.

The pea-wilt fungus can infect the pea plant and produce the disease without the aid of other microörganisms or of gross mechanical injury. Peas grown in cotton-plugged culture tubes of sterilized soil or of an agar substratum inoculated with a pure culture of the pathogene develop the symptoms readily. Wounding the root system delays slightly, rather than hastens, the appearance of symptoms in plants of a susceptible variety but does not lower the degree of resistance of resistant peas.

Pea wilt develops more rapidly in soil heavily infested with the wilt fungus than in lightly inoculated soil. Dilution of highly infective soil with steam-sterilized soil leads to greater retardation of the disease than a corresponding dilution with noninfested raw soil.

A search for soil toxicity developed by the action of the wilt fungus has given only negative results.

In the characteristic development of pea wilt under conditions favorable for the disease, there are very distinctive and significant changes in the affected plants which precede wilting or leaf necrosis. The course and degree of some of these changes are considered to provide a key to probable

factors in pathogenesis. Pronounced dwarfing is accompanied by increased rigidity of the entire shoot, hypertrophy of the lower stem internodes, and rolling of leaf laminae. Before wilting begins, diseased pea stems lose water more slowly upon exposure to drying and have a higher content of dry matter with a proportionately increased ash content, an increased osmotic value of the cell sap, and an increased capacity for regeneration. These symptoms do not indicate general starvation or drouth of the plant as a whole but they do suggest unbalanced nutrition and failure of translocation.

Filtrates from Richards' Solution cultures of the wilt fungus produce a type of rapid necrosis in cut pea stems which appears comparable to the sudden wilting of young plants in the early development of this disease. Varieties of peas differ in susceptibility to toxic culture filtrates, but experiments with 10 strains and varieties have failed to reveal any correspondence between this and the resistance or susceptibility of these same varieties to the Fusarium wilt.

A shortage of water induced directly or indirectly by the fungus may sometimes be a factor in the wilting of large plants but probably not in the early collapse of young seedlings. In the main, pathogenesis must be attributed to the action of toxic substances resulting from the presence of the wilt fungus within the host plant; but this is a far more complex and systemic action than generally conceived, leading first to a derangement of metabolic and growth processes and finally to death of the tissues. Actual wilting or slow necrosis, as the case may be, comes as the culmination of a long series of changes induced by the influence of the fungus.

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WOUND INOCULATION IN RELATION TO RESISTANCE IN THE FUSARIUM WILT OF PEAS[1]

MAURICE B. LINFORD

INTRODUCTION

In the course of histological studies of resistance and susceptibility of peas (*Pisum sativum* L.) to the wilt disease caused by *Fusarium orthoceras* App. and Wr. var. *pisi* Linford, it became desirable to gather experimental evidence concerning the possible localization of the expression of resistance. The writer showed earlier (1) that varietal resistance to this disease is sharply defined, approaching very close to immunity even under conditions most favorable for the disease in susceptible varieties of peas. Wade (5) has since presented further evidence that inherent resistance is essentially complete. The writer's tests with toxic culture filtrates (2) showed that resistance in this case is not a question of tolerance by the resistant plants for the products of fungal metabolism. Preliminary histological investigations (1) had indicated that the fungus failed to establish itself in the vascular system of the resistant plants and suggested that resistance is expressed chiefly in the root system. It appeared probable that if the wilt fungus could be introduced directly into the vascular system of the aerial shoot by some mechanical means, the disease might develop in resistant as well as in susceptible plants. This was attempted through the inoculation of wounds in stems and petioles which exposed truncated vascular bundles to the pea-wilt fungus.

EXPERIMENTAL

Peas of the Badger (susceptible) and Horal (resistant) varieties, grown 10 days from seed to the 4-node stage in pots of sterilized soil, were inoculated as indicated in table 1. As soon as a plant was inoculated it was wrapped in wet absorbent cotton and gauze, and, when all plants in a pot were ready, additional cotton was applied; the whole was wetted thoroughly and placed in a moist chamber where it was kept wet for 40 hours. Bandages were then removed and the pots were placed in the open greenhouse for the remainder of the experimental period of 1 month. Fifteen plants of Badger and 15 of Horal were inoculated in each way, and 5 of each were

[1] This paper is one of a series reporting studies on pathogenesis and resistance in the Fusarium wilt of peas conducted under a fellowship appointment from the National Research Council. The writer wishes again to express his appreciation to the Department of Botany, University of Wisconsin, for the facilities extended, and to Professor B. M. Duggar for helpful criticism throughout this work.

TABLE 1.—*Results of wound inoculation experiment with Fusarium orthoceras var. pisi and both wilt-resistant and susceptible varieties of peas. Data taken after 1 month*

Method of inoculation	Badger variety (susceptible		Horal variety (resistant)	
	Number infected[a]	Per cent	Number infected[a]	Per cent
1. Tangential slice cut in cortex of second stem internode and barley-grain culture inserted	0	.....	1	6.7
2. First petiole excised at its base and barley-grain culture applied to wound	2	13.0	3	20.0
3. First petiole excised at its base and Richards' solution culture applied to wound	0	.....	0	.....
4. Stem tip removed above fourth node and barley-grain culture applied to wound	1	6.7	2	13.3
5. Not wounded. Barley-grain culture placed on soil	0	.....	0	.....

[a] Fifteen plants each of Bodger and Horal peas were inoculated by each method. Five plants of each variety were wounded and left as uninoculated controls. These controls all remained healthy. Details of technique are given in the text.

held as controls, with the plants wounded but not inoculated. Additional controls were provided by spreading inoculum on the surface of the soil around non-wounded plants and by the omission of all wounding and application of the fungus.

All the control plants remained healthy and recovered from the wounding without conspicuous reaction or loss of vigor. Of the inoculated plants a small percentage became infected, and these were all from the barley-culture inoculation. Of the 45 plants of each variety, 90 plants in all, inoculated through wounds with the culture growing on cooked barley kernels, 3 plants of the Badger variety, or 6.7 per cent, and 6 of the Horal, or 13.3 per cent, became infected as indicated by the development of symptoms.

The symptoms developed by these plants were closely similar to those typical of the wilt disease but differed somewhat both in details of appearance and in sequence of development. Response became apparent in

Badger and Horal alike a few days after inoculation and developed rapidly to its maximum expression, which varied from plant to plant. Growth of the terminal bud was retarded or completely checked, stems and petioles became swollen and rigid, and leaflets and stipules rolled backwards or became distorted, thick, firm, and darker green than normal. The whole plant assumed the condition of extreme rigidity, typical of this disease before wilting begins, but showed none of the general yellowing and the excess development of waxy bloom which accompany its development from natural infection through the roots. After this quick reaction, further changes occurred slowly. In some plants of both varieties, stipules, leaflets, and petioles, and even the stem tips collapsed in a manner typical of severe development of the disease. One plant of each variety made partial recovery, resuming apical growth with the production of approximately normal leaves. The other affected plants neither recovered nor developed more severe symptoms during the remainder of the experiment.

HISTOLOGICAL FEATURES

Microscopic examination of infected plants of both varieties revealed a condition different from the typical pathological histology of pea wilt but with many points of essential similarity. Free-hand sections and serial paraffin sections were prepared from the infected plants and from several healthy control plants. In the controls there was little vascular discoloration or plugging of the vessels with gum-like materials, and this only in or near the actual wounds. Inoculated plants which had not become infected showed similar but somewhat greater disturbances. In all plants which showed symptoms of the disease, however, the fungus was present abundantly in the margins of the wounds and extended variable distances into the connecting vascular bundles.

Even in the most severely damaged plants there was no general invasion of the vascular system. The fungus remained localized within those vascular bundles which it entered first and was apparently limited in its vertical extent by the occlusion of vessels with products of wound reaction. In the single plant infected from a cortical wound the fungus was found in the cortex and in both stipule traces; in plants inoculated through cut petioles it extended chiefly downward through the leaf trace; and in plants inoculated through the stem tip it extended downward along several bundles. In most plants it was limited to a vertical extent of a few millimeters from the wound, and in no case was it seen to have traversed more than two internodes from the point of inoculation.

Such local development of the fungus within a vascular bundle had stimulated the surrounding parenchymatous cells, leading to the formation

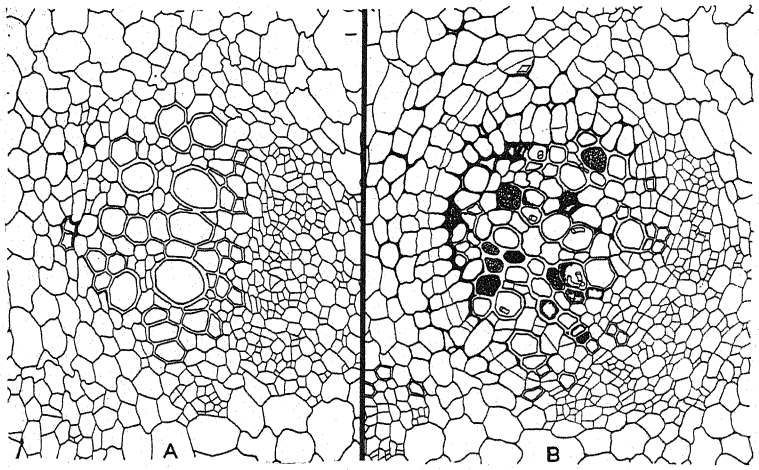


FIG. 1. Leaf traces of Horal (R) pea plant inoculated with the pea-wilt fungus through a cut petiole at the third node. This plant had stood one month following inoculation before the material was fixed for sectioning; the stem was swollen and the leaflets were all collapsed. Beyond the parenchyma immediately in the margin of the wound the fungus was present only in the leaf trace of the inoculated petiole. Drawn with the aid of a camera lucida,× 203. A. Leaf trace free from visible disturbance, from the upper part of the third internode, below the inoculated petiole but from the opposite side of the stem. B. Infected leaf trace from the same level in the third internode. This vascular bundle, which extends into the inoculated petiole, illustrates varied responses to invasion of the vessels, including accumulation of gum-like and granular deeply staining materials in the vessels; altered staining reaction of walls, particularly at the margin of the xylem tissues; the formation of a periderm-like sheath surrounding the xylem, through both hypertrophy and hyperplasia of the parenchyma; and a mild hypertrophy of meta- and secondary phloem.

of a cambium-like layer and the development of a sheath of new tissue several cells in thickness surrounding the xylem portion of the bundle. This occurred both in the central cylinder (Fig. 1, B) and around the stipule traces in the cortex (Fig. 2, B). In some instances these newly formed cells enlarged greatly, crowding the normal surrounding tissues markedly out of place.

It was demonstrated clearly in this study that even a small amount of the fungus within the plant is sufficient to produce symptoms of this disease. In the single Horal plant which became infected from inoculation through a cortical wound (Fig. 2) the leaflets, stipules, petioles, and even the tip of the stem wilted. Serial sections from a point well above the lesion to a point far below showed that in no place had the fungus entered the central cylinder. It was present only in the cortical parenchyma at the margin of the wound and in the two stipule traces through which it had

extended a few millimeters up and down the stem. In such a case it seems clear that the development of preliminary symptoms and finally the collapse of leaves and succulent parts of the stem were stimulated by the action of the wilt fungus at a distance, acting through some toxic substances which became distributed throughout the plant.

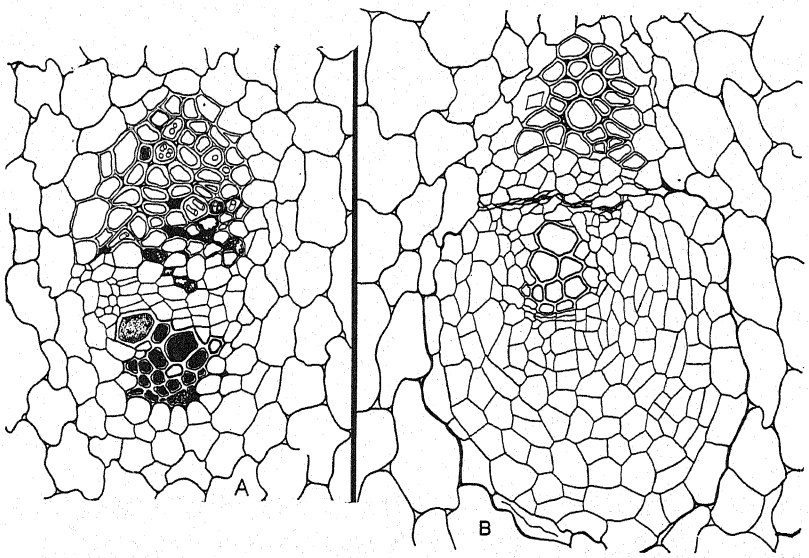


FIG. 2. Stipule traces of Horal (R) pea plant inoculated with the pea-wilt fungus through a cortical wound in the second internode. This plant had stood one month after inoculation and was in late stages of collapse at the time this material was fixed for sectioning, but infection was limited to these two stipule traces and the margin of the cortical wound. Drawn with the aid of a camera lucida, ×290. A. Occlusion of xylem vessels and invasion of phloem fibers of stipule trace just below the lower limit of cortical invasion at the margin of the inoculation wound. Except the one large vessel with granular contents, all vessels in this bundle were completely occluded with gum-like material at some level, although not all at one level. Invasion of the phloem and phloem fibers was accompanied by necrosis and an accumulation of deeply staining matter in and between the cells. Compare with the nonnecrotic condition of B. B. The other stipule trace from a section through a lower level of the same internode, showing a condition of extreme hyperplasia of the parenchyma surrounding the xylem. Closer to the wound this vascular bundle was largely occluded and still closer was thoroughly invaded by the wilt fungus. Note the cambium-like activity immediately at the base of the protoxylem. The metaphloem has been crushed by this hyperplastic tissue.

Occlusion of xylem vessels with brown, granular or more typically gum-like, deeply staining materials was a conspicuous feature of this infection and apparently was a major factor in the very limited longitudinal distribution of the fungus along the vessels. Gum-like material (Fig. 2, A) was present in the vessels not only in immediate association with the fungus

but also at a point well in advance. These materials, apparently secreted into the lumina of the vessels by the xylem parenchyma, appeared to constitute a barrier to the progress of this fungus. Near the wound where the fungus was abundant in the vessels, the xylem parenchyma cells were chiefly dead and wound gums were present only sparingly; farther from the wound, where the fungus was less abundant and the parenchyma still alive, such materials were relatively more abundant; and still farther away, beyond the fungus, they were seen to plug some or all the vessels of an infected bundle (Fig. 2, A). Substances of similar appearance were seen in and on the walls of the parenchyma cells damaged by the fungus, both cells of the primary tissues and cells formed in response to this invasion. As will be shown in a following paper, this appears directly comparable with some observed host reactions in the normal development of the wilt disease in susceptible plants. Likewise it resembles certain more pronounced responses observed in the tissues of resistant pea plants grown in infested soil.

DISCUSSION

The low percentage of infection obtained in this work is in agreement with the experience of Tisdale with flax wilt (4) and indicates that the pea-wilt fungus is not an aggressive invader of wounds. Tisdale, however, obtained no infection of resistant plants. The writer (2) and Wade (5) failed to find evidence of invasion of pea roots through wounds by the pea-wilt fungus. This is indicative of a specialized parasitism on the part of the fungus and also of a mild degree of resistance to the invasion of aerial parts even in the susceptible variety of pea. The pronounced host response, comparable with some resistance reactions, which apparently served in this case to check advance of the fungus, is further evidence that both varieties alike are resistant to the wilt fungus when it is thus introduced without any of the preliminary alterations in the host physiology which typify the development of pea wilt from the usual root infection (2).

The symptoms that developed in the infected plants were as closely similar to the characteristic symptoms of pea wilt as might be expected and were the only close approach to the symptoms of this disease that the writer has seen in Horal peas. Since the fungus was sharply limited in its development and became completely shut off after a time by the host responses, the complete sequence of symptoms could not be expected, particularly since, in this type of inoculation, the root system remained free from injury.

In spite of the indication of some degree of resistance to the fungus in the shoots of resistant and susceptible peas alike, these results agree with the writer's tests with toxic culture filtrates (2) in indicating that the

sharply defined varietal resistance is not a question of tolerance of toxic products of the wilt fungus, since the resistant and susceptible peas reacted essentially alike in the present case. Moreover, these results indicate that varietal resistance is expressed locally within the root system, probably during the attempted entry of the wilt fungus into the vascular system, as will be considered in the following paper. This is supported by May (3) who reports, in work published since the completion of this study, that the tomato-wilt Fusarium, introduced into the shoot of a resistant plant through a graft union with a susceptible plant, is able to advance and produce wilt symptoms.

SUMMARY

By inoculation of young pea plants with *Fusarium orthoceras* App. and Wr. var. *pisi* Linford through wounds in aerial parts, symptoms were obtained which partially simulated those typical of the pea-wilt disease. A higher percentage of infection was obtained in this way in the resistant variety, Horal, than in the susceptible variety, Badger, and the course of the resulting disease was similar in both varieties. These results are taken to indicate that the expression of varietal resistance to the pea-wilt disease is largely localized within the root system.

EXPERIMENT STATION, ASSOCIATION OF
HAWAIIAN PINEAPPLE CANNERS,
HONOLULU, HAWAII.

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HYBRIDIZATION AND SEGREGATION IN THE OAT SMUTS

C. S. HOLTON[1]

INTRODUCTION

The discovery of physiologic specialization in smut fungi has stimulated investigations to determine whether or not physiologic forms arise by hybridization. Theoretically, new physiologic forms may arise either through hybridization between different forms or between different species, as well as by mutation. The writer has made studies to determine whether the two species of oat smuts, *Ustilago avenae* (Pers.) Jens. and *Ustilago levis* (K. and S.) Magn., hybridize.

REVIEW OF LITERATURE

Kniep (9) has shown that different species of smut will hybridize in culture to the extent that sporidia of opposite sex will fuse. Dickinson (3) has made histological studies which show that *Ustilago levis* and *U. hordei* (Pers.) K. and S. in combination produced infection in oats, whereas monosporidial lines alone produced no infection. However, he does not state that the infection referred to above resulted in the production of chlamydospores.

Kämmerling (8) obtained infection with crosses between monosporidial lines of opposite sex of *Ustilago longissima* (Sow.) Tul. and *U. longissima* var. *macrospora* Davis and found that similar hybrids occur in nature. Furthermore, Hanna and Popp (7) have obtained chlamydospore production by inoculating oat seedlings with crosses between monosporidal lines of *U. avenae* and *U. levis*.

Dickinson (4) has studied the nature of segregation within *Ustilago levis* and within *U. hordei*. He found that segregation for cultural characters was on a 2:2, 3:1, and 4:0 basis and that this segregation may take place in either of the "reduction divisions." Dickinson (2) found also that the segregation for sex factors in *U. levis* as well as in *U. hordei* was on a 2:2 basis, and Hanna and Popp (7) obtained similar results for *U. levis* and for *U. avenae*. That the segregation for sex factors is independent of the segregation of factors for cultural characteristics has also been demonstrated by Dickinson (4), and he was the first to show (5) that the nuclear division in which segregation occurs may be affected by alteration of the environmental conditions.

[1] The writer is greatly indebted to Dr. E. C. Stakman for suggesting the problem and to Dr. J. J. Christensen for many suggestions offered during the course of the investigations.

Stakman, Christensen, Eide, and Peturson (10) pointed out that if the numerous variants they obtained in *Ustilago zeae* (Beckm.) Ung. were not the results of mutations, they probably were due to some abnormal type of segregation. Christensen (10, Part II) obtained some indication of delayed segregation in certain self-fertile lines of *U. zeae*.

HYBRIDIZATION

In September, 1930, a large number of primary sporidia were isolated from the promycelia of germinating chlamydospores of *Ustilago avenae* and *U. levis,* and of this number 4 that were isolated from *U. avena*e and also 4 from *U. levis* were cultured on 1.3 per cent potato-dextrose agar. The 8 monosporidial lines thus secured, comprising 2 species, were paired in all possible combinations on nutrient-free agar and observed for fusions. The segregation for sex factors, as indicated by sporidial fusions, was found to be on a 2:2 basis with perfect interspecific fertility.

Pathogenicity tests were made by inoculating Anthony oats seedlings with intra- and interspecific crosses. Inoculations were also made with monosporidial lines and with combinations of monosporidial lines of similar sex, as indicated by the absence of sporidial fusions in culture. Plants that were not inoculated served as checks.

The inoculations were made in the following manner. The various monosporidial lines and crosses were cultured on nutrient-free agar in Petri dishes for 2 days. Ten cc. of sterile distilled water was then added to each culture, forming a suspension of the inoculum. Oat seedlings were placed in the inoculum and incubated at 18° to 20° C. for 48 hours, after which time they were transplanted to pots and incubated 6 days at the same temperature. The plants were then removed to the greenhouse where they grew to maturity. The results of the inoculations were obtained in November, 1930, and are summarized in table 1.

It is clearly evident from the data presented in table 1 that *Ustilago avenae* (spores echinulate) and *U. levis* (spores smooth) hybridize readily, as evidenced by the production of chlamydospores on the host. All combinations of monosporidial lines in which fusions were observed in culture produced chlamydospores on the host, the percentages of smutted panicles ranging from 23.8 to 70.5 in the interspecific crosses and from 18.8 to 72.7 in the intraspecific crosses. No smut was produced by inoculation with cultures of monosporidial lines alone or with combinations of monosporidial lines which did not fuse in culture.

Microscopic examinations revealed the fact that the interspecific hybrid chlamydospores were echinulate, while the intraspecific hybrid chlamydospores had markings characteristic of the species. Thus, echinulation is

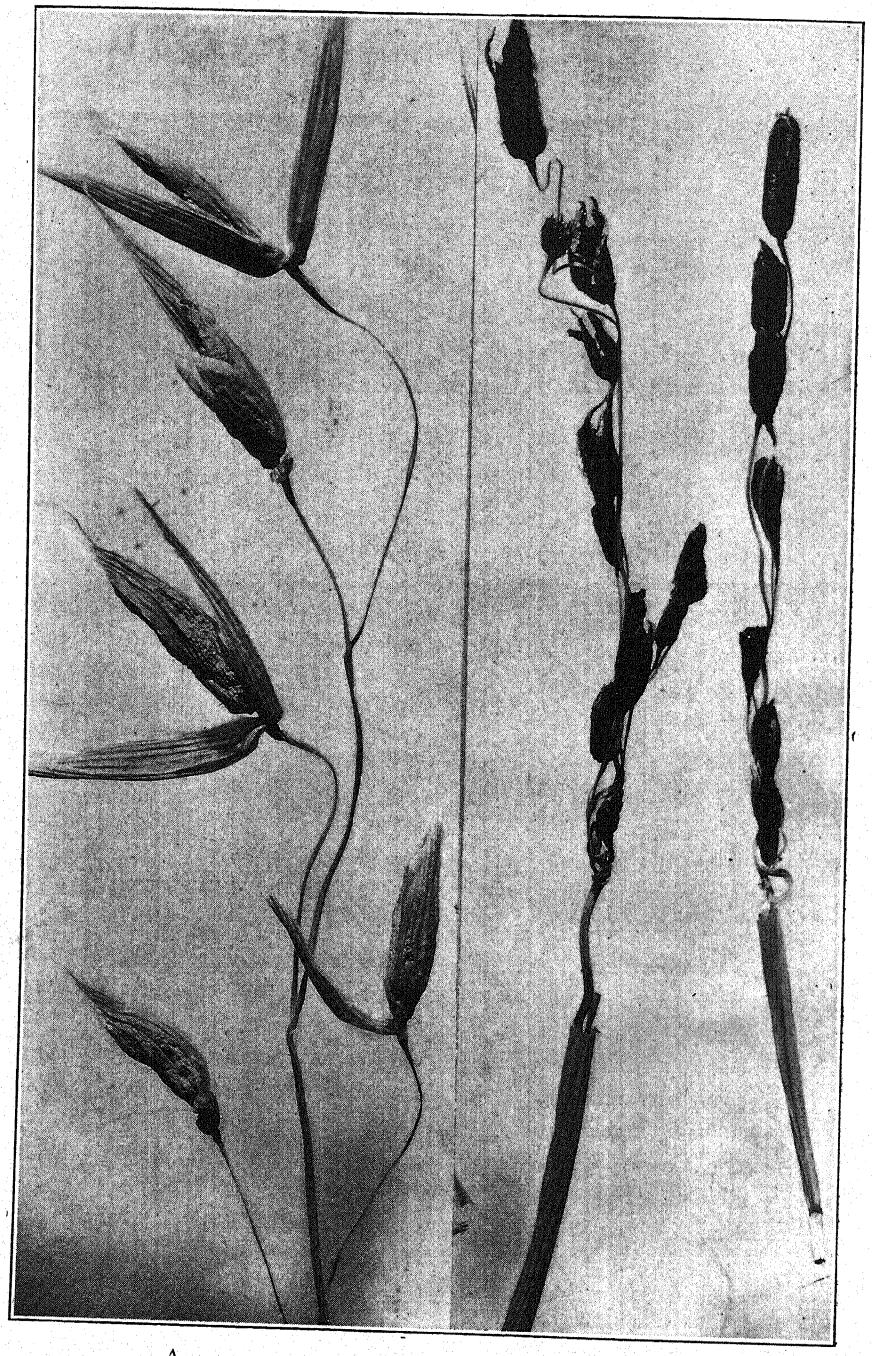
TABLE 1.—*The results of inoculating Anthony oats with intra- and interspecific crosses between monosporidial lines of Ustilago avenae and U. levis*

Lines and crosses	Number of seedlings inoculated	Number of panicles smutted	Percentage of smut
U. avenae			
1	20	0	0.0
2	25	0	0.0
3	32	0	0.0
4	30	0	0.0
1 × 3	22	16	72.7
2 × 4	28	5	18.8
2 × 3[a]	21	0	0.0
U. levis			
1	20	0	0.0
2	24	0	0.0
3	30	0	0.0
4	28	0	0.0
1 × 4	24	5	20.8
2 × 3	26	6	23.0
3 × 4[a]	27	0	0.0
U. avenae × *U. levis*			
1 × 3	34	24	70.5
2 × 1	24	12	50.0
4 × 3	21	5	23.8
4 × 4	21	12	27.1
Check	75	0	0.0

[a] Combinations in which sporidia did not fuse in culture.

apparently dominant over smoothness, in this case at least. Furthermore, the smutted panicles on the host were of the "loose" type in all cases of interspecific hybrid infection, although there was considerable variation in degree. Usually this type of injury can be attributed to *Ustilago avenae,* the species with echinulate spores. The intraspecific crosses produced the loose type of smut in the case of *U. avenae* and the covered type of smut in the case of *U. levis.* These results agree with those recently reported by Hanna and Popp (6).

Germination tests were made and it was found that the interspecific hybrid chlamydospores germinated and produced primary sporidia in a normal manner, but, when these sporidia were isolated, they failed to develop, except for occasional ones. More than 300 of these sporidia were isolated and only 5 cultural lines were obtained, representing both sexes.



A B

FIG. 1. Two types of oat smuts showing difference in color on the host.
A. Buff type. B. Normal, dark type.

Thus, in view of the fact that the primary sporidia isolated from germinating intraspecific hybrid chlamydospores developed normally in culture, it appears that some lethal factor or factors may be present and become effective in certain interspecific crosses. Whether this fact is generally true with all crosses between *Ustilago avenae* and *U. levis* has not been determined. Several different lines of *U. levis* have been crossed with the same line of *U. avenae* and in every case isolated sporidia failed to develop. It is entirely possible, if other lines of *U. evenae* were used, that the sporidia from some of the interspecific hybrid chlamydospores would develop normally.

For testing the pathogenicity of the interspecific hybrids 2 monosporidial lines of opposite sex have been crossed with each other and back-crossed with the parent lines. The back-cross with the *Ustilago avenae* parent produced infection (25 per cent) on Liberty Hulless oats and the chlamydospores were smooth, while the back-cross with the *U. levis* parent produced no infection on the same variety. The cross between the 2 monosporidial lines, originating from interspecific hybrid chlamydospores, produced infection (30 per cent) on Liberty Hulless oats and in this case the smut on the host was buff instead of black, the normal color for oat smuts. When examined microscopically, it was found that the individual chlamydospores were smooth and apparently colorless. They germinated normally and when sporidia were isolated from the promycelia these

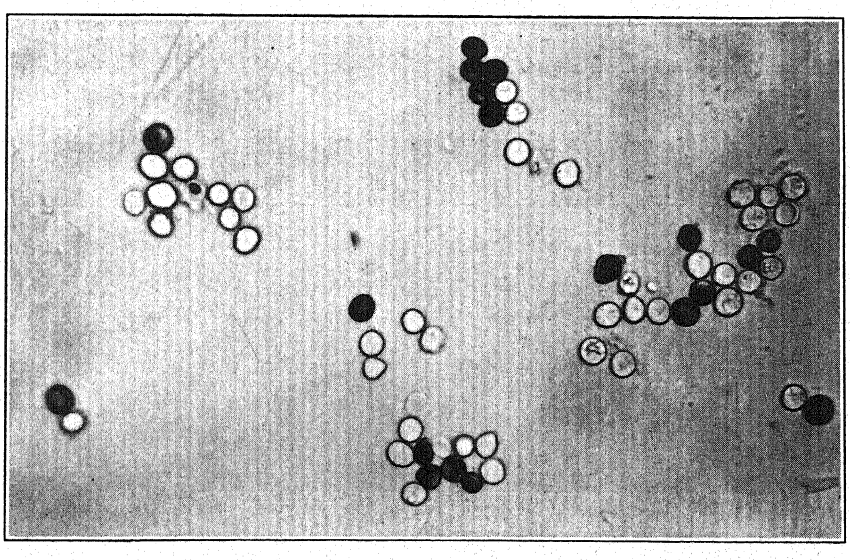


FIG. 2. Photomicrograph showing the contrast in color between the individual chlamydospores of the buff type of oat smut and the normal dark type.

sporidia reproduced normally in culture. The striking contrast between the buff and the normal type of oat smut on the host is shown in figure 1, while the contrast between the color of individual chlamydospores of the 2 types of smut is shown in figure 2. The pathogenicity of the buff type of smut has not been determined. Campagna (1) has described a buff type of loose smut of wheat occurring in nature.

SEGREGATION

The nature of segregation for sex factors and cultural characteristics has also been studied. All 4 primary sporidia have been isolated from a number of germinating chlamydospores by the method described by Hanna (6). As has already been stated, the segregation for sex factors was found to be on a 2:2 basis. Furthermore, all possible arrangements of these sexes on the promycelium have been found, indicating that reduction for sex may take place in either the first or second division of the reduction process.

Segregation of factors for cultural characteristics, such as color, topography, type of growth, and rate of growth, takes place in either division of the reduction process of *Ustilago avenae* and *U. levis* and segregation of these factors is independent of segregation for sex factors. Dickinson's work (4) has shown the same to be true for *U. levis*.

Successively produced sporidia were isolated from the same segment of the promycelium in order to determine whether or not further segregation occurs in the individual segments. If further segregation does not occur in the segments of the promycelium, then successively produced sporidia should develop lines that are identical in their cultural and sexual reactions.

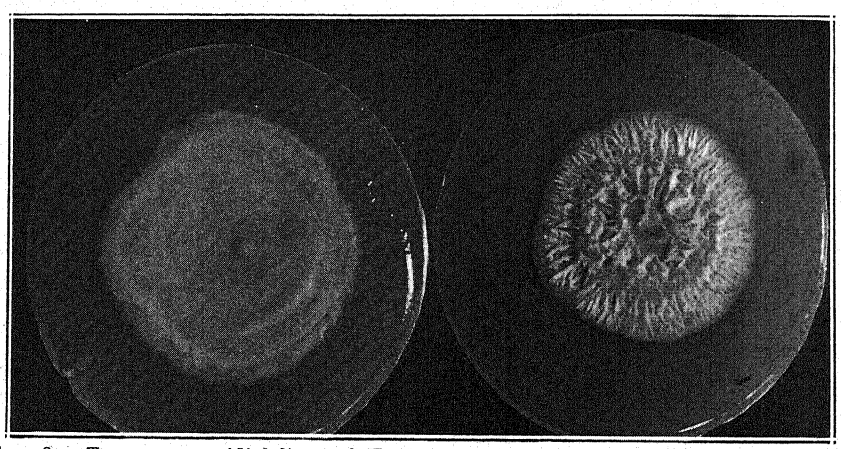


FIG. 3. Two monosporidial lines of *Ustilago avenae* obtained by isolating successive sporidia from the third segment of the promycelium of a germinating chlamydospore.

As many as 5 successive sporidia have been isolated from 1 segment and 2 to 3 successive ones from a number of other segments of the same promycelium and of different promycelia. Cultural comparisons of these isolations were made on 1.3 per cent potato-dextrose agar. Of the 5 monosporidial lines originating from the basal segment of 1 promycelium 4 were different in their cultural characteristics. In the majority of cases where 2 or 3 monosporidial lines originated from the same segment they were quite distinctly different in cultural characteristics. Typical cultural differences between lines originating from the same segment of the promycelium are



FIG. 4. Two monosporidial lines of *Ustilago levis* obtained by isolating successive sporidia from the second segment of the promycelium of a germinating chlamydospore.

clearly exhibited in figures 3 and 4. Thus, it seems quite evident that segregation for certain cultural characters is delayed beyond the second division of reduction division in *Ustilago avenae* and *U. levis*. All monosporidial lines originating from the same segment of the promycelium were of the same sex. Therefore, it appears that segregation for sex factors usually takes place in either the first or second division of reduction division and not in subsequent divisions as it does for other characters.

SUMMARY

1. *Ustilago avenae* and *U. levis* are perfectly interfertile. Monosporidial lines of opposite sex fuse in culture and also produce smut on the host, regardless of whether crosses are made inter- or intraspecifically.

2. Sporidial fusions do not occur within a monosporidial line of either species and neither do they occur in intra- and interspecific combinations of monosporidial lines of similar sex. Furthermore, when such combinations are used for inoculation, no smut is produced on the host.

3. Intraspecific crosses produced the loose type of smut in the case of *Ustilago avenae* and the covered type in the case of *U. levis* while the smut produced by the interspecific crosses was of the loose type.

4. Intraspecific hybrid chlamydospore markings were characteristic of the species, but the interspecific hybrid chlamydospores were echinulate.

5. Primary sporidia isolated from the promycelia of germinating interspecific hybrid chlamydospores would not develop in culture, with rare exceptions. However, primary sporidia isolated from the promycelia of germinating intraspecific hybrid chlamydospores developed in a normal manner.

6. A cross between two monosporidial lines that originated from interspecific hybrid chlamydospores produced a buff type of smut which is apparently a new and previously undescribed type. The chlamydospores of this smut were smooth and apparently colorless, in contrast to the dark brown chlamydospores of the common type of oat smut.

7. Segregation for sex factors in *Ustilago avenae* and *U. levis* was found to be on a 2:2 basis. There is strong evidence that a delayed segregation of factors for certain cultural characteristics occurs in *U. avenae* and *U. levis* since monosporidial lines originating from the same segment of the promycelium often exhibit striking differences in cultural characteristics.

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THE ANTIBIOSIS OF CERTAIN BACTERIA TO SMUTS AND SOME OTHER FUNGI[1]

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INTRODUCTION

In the course of some investigations on corn smut in 1926 and 1927, Bamberg (1) found that gall production often failed to occur upon susceptible plants when these were inoculated with the proper combinations of corn-smut lines of known virulence. In a large percentage of cases only a discolored area was produced at the point of inoculation. From such discolored areas bacteria were frequently isolated that were distinctly antibiotic to several species of smuts.

Bamberg found that the bacteria destroyed colonies of *Ustilago zeae* (Beckm.) Ung. of 9 days' growth when he transferred a small amount of the bacterial culture to the edge of smut colonies, the latter being reduced to a slimy mass with only a few sporidia present. In hanging-drop preparations of both the sporidia and the bacteria the former were destroyed in a few days. He also found that the bacteria checked further development of the smut galls on corn plants when he inoculated a suspension of them into the galls with a hypodermic syringe. Inoculation with corn-smut cultures in combination with the bacterial culture usually did not result in gall formation in corn plants. The bacteria also destroyed colonies of *U. zeae*, *U. avenae* (Pers.) Jens., *U. levis* (K. & S.) Mag., *Tilletia tritici* (Bjerk.) Wint., and *Sorosporium reilianum* (Kühn) McAlp. This bacterial culture was given to the writer to determine, if possible, what factors might be responsible for the phenomenon. The writer had also isolated three cultures of bacteria that were antibiotic to several smuts and certain other fungi. This paper deals with the investigations on these cultures. Bamberg called his culture B–1, and the same designation will be used in this paper. The other three cultures are referred to as C–1, D, and *Myxobacterium*–1.

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[2] The writer wishes to express her indebtedness to Dr. E. C. Stakman, who suggested the investigation and offered valuable suggestions. She also acknowledges helpful criticism from Dr. Leach and many favors from M. B. Moore, J. M. Walter, and R. H. Bamberg of the Plant Pathology Department.

EXPERIMENTAL WORK ON BACTERIAL CULTURE B–1

Bamberg's culture consisted of a coccus and a rod-like bacterium. He was not certain whether he had a pleomorphic organism or a mixed culture, which could not be purified by repeated plating on account of the viscosity of the growth. The writer also was unable to separate the two bacteria by plating. However, the rod-like form had a higher thermal death point than the coccus and was obtained in pure culture by heating the mixed culture slightly above the thermal death point of the coccus. In the course of the filtration experiments mentioned later, a coarse filter was used through which the coccus, but not the rod-like form, passed. In this way a pure culture of the coccus was obtained. It was soon found that the coccus was the antibiotic organism and the rod-like form had no effect upon sporidial growth. The designation B–1 is therefore retained for the coccus culture in this discussion.

The writer repeated some of Bamberg's experiments and found that the sporidia were destroyed in about two weeks in large colonies of *Ustilago zeae, U. avenae, U. levis,* and *Sorosporium reilianum,* when these were inoculated at the edge with the bacterial culture. The sporidia of the smut colony were gradually replaced by a slimy mass of bacteria. The same result was obtained when a small amount of the bacterial culture was placed on top of large colonies of the smut. The sporidia were destroyed also in hanging drops prepared from bacterial and sporidial suspensions in tap water, the bacteria increasing greatly in number, while globules and débris were observed in increasing quantities in the preparation. When the bacterium was inoculated into corn plants in combination with the proper sporidial lines of smut, the plants often failed to develop galls, although galls were present in the checks. When it was inoculated with a hypodermic syringe into galls, these frequently shriveled up, while galls inoculated with sterile water were not so affected. The effect of the bacteria was more marked on young galls than on older ones, the membrane of the former often turning green, all traces of the gall being obliterated as the growth of the plants progressed. The mature galls generally dried up, but the host tissue had been replaced by the fungus tissue to such an extent that chlorophyll production did not occur. The inoculation with sterile water and with a water suspension of the bacterium without sporidia had no effect upon corn plants. In another series of experiments the bacterium seemed to have very little effect upon the various smuts.

These variations in the antibiotic effect of the bacterium seemed rather puzzling. However, two explanations suggested themselves. One might be loss of virulence under certain conditions. The bacterial cultures used, especially in the second series, had been cultivated for some time upon artificial media, and this might have affected the virulence. Checks were made

to determine this, and the results showed that when the bacterium had been cultivated for several months upon artificial media, it was no longer able to attack the smut cultures nor to inhibit gall formation in corn plants. In some of the stock cultures the virulence could not be revived, but in others it was "stepped up" by successive inoculations simultaneously with small amounts of smut growth into media suitable for the growth of both. When such bacterial cultures were combined with the proper sporidial lines and inoculated into corn plants, many of the plants showed no infection, although galls formed in the checks. Another explanation of failure of the bacteria to inhibit gall formation in corn plants might be that the sporidia were present in preponderating numbers in the inoculum and that the bacteria could not multiply fast enough to prevent infection. It is obvious that the survival of only two sporidia of proper affinities may result in the infection of a plant. To check on this possibility, varying amounts of the bacterium were combined with constant amounts of the sporidia and inoculations were made from these combinations into corn plants. Inhibition of gall formation occurred constantly when a highly concentrated suspension of an active culture of the bacterium was used. The failure of the bacterium in the earlier experiments to show consistently properties antibiotic to smuts was probably due to a loss of virulence from prolonged cultivation upon artificial media as well as to a perponderance of viable smut sporidia in the inoculum. Failure of the bacterium to prevent gall formation in the corn plants does not necessarily indicate a lack of antibiotic ability under proper conditions.

The bacterium seems to have no effect on old smut cultures. At first this was thought due to the dryness of the smut culture, but the addition of sterile water at the time of inoculation with the bacterium did not increase its activity.

As was found by Bamberg, the organism is most active at fairly high temperatures, 20° to 25° C., although it grows at 10° C. and retains its antibiotic property. No attempt was made to grow it at lower temperatures.

Very little is known about the way in which bacteria may attack fungi. It is evident that the action upon the fungus is either extracellular entirely or extracellular at first and intracellular later. Tolaas (22) and Paine (14) found a fluorescent bacterium that produces disfiguring spots upon cultivated mushrooms. But neither of these two investigators made any study of the mode of attack of this bacterium. Petri (15) of Portici, Italy, in 1927, described a bacterium that penetrates and destroys the hyphae of Phytophthora and Pythiacystis species. Similarly, Sanzone (17) of the same station reported a bacterium which lives symbiotically within the hyphae and conidia of *Fusarium solani* (Mart.) Sacc. Geitler (7) found a bacterium, parasitic upon Cladophora, which destroys the alga by pene-

trating the cells. However, neither Bamberg nor the writer has been able to find any indication that bacterium B–1 enters the hyphae or the sporidia of the smut. This suggestes that the active principle is extracellular and capable of breaking down the cell wall. Such a substance might be an enzyme. Therefore, a study of the enzymes of the bacterium and an analysis of the chemical constituents of the cell wall of the sporidia seemed necessary.

Considerable work has been done on the composition of the cell wall of the lower Thallophytes. A recent work on the subject is by Wettstein (24), who also summarizes the work of other investigators in this field. According to their findings, the Zygomycetes, one of the subgroups of the Phycomycetes, contain chitin in the cell wall; and the Oomycetes, the other subgroup, contain cellulose. In the Ascomycetes chitin has been found to be the principal constituent, but other substances are often present. Wettstein makes the following statement in regard to the Basidiomycetes: "Die Basidiomyceten sind, was das Vorkommen von Chitin betrifft, gleichfalls einheitlich. Es ist auch hier die überwiegende Membransubstanz in fast allen Gruppen. Bei manchen treten wieder andere Substanzen hinzu, die neben Chitin vorhanden sind, oder auch dieses ersetzen können. Cellulose fehlt vollständig. Bei abgeleiteteren Gruppen, Polyporeen, Gasteromyceten, scheint die Zahl der verschiedenen Membransubstanzen sehr gross zu sein." Wettstein makes no mention of smuts and rusts. It is probable that no tests were made on these groups. Microchemical tests for the presence of chitin and pectin in the cell wall of corn smut were therefore made by the writer.

CHEMICAL COMPOSITION OF THE CELL WALL OF CORN-SMUT SPORIDIA

Chitin test. The test for chitin is made by boiling the material in concentrated KOH for about 30 minutes to change the chitin to chitosan. It is then washed in 95 per cent alcohol to harden, and a solution of IKI, the reagent for chitosan is added. A red violet color indicates the presence of chitosan.

When so treated portions of individual sporidia stain red while the remainder of the sporidium is colorless. The red violet areas indicate the presence of chitin; and the colorless areas some other chemical constituent. The stained sporidial mass is red, possibly indicating a preponderance of chitin.

Pectin tests. A. Ruthenium red. A small amount of sporidial material was placed on a slide and a dilute solution of Ruthenium red (1–10,000) was added, the slide being kept in the dark. After about 20 minutes the reagent was washed off. A red color appeared in the sporidial mass and in patches in the individual sporidia. This reaction indicates the presence of pectin in scattered areas of the cell wall.

B. Methylene blue. Pectic substances stain violet with methylene blue (1–1,000), and cellulose usually stains blue. When methylene blue was added to the sporidial material on the slide, a violet color occurred in patches on the walls of the sporidia, other areas being blue. The violet reaction is more marked in young sporidia; and the blue, in the old. This result suggests the presence of pectin and possibly other materials such as cellulose or related substances, in the cell wall of the sporidia. These findings are somewhat in accord with Wettstein's statement that in some groups the number of different membrane substances seems to be very large.

TESTS FOR ENZYMES IN BACTERIUM B–1

Filtration experiments. Bamberg found that the addition of a small amount of the filtrate of a broth culture of B–1 had no effect upon corn smut. The writer filtered both fresh and old broth cultures of the bacterium with a fine filter, which eliminated the bacterium. Smut always grew readily in it. This was repeated many times with the same results. It indicates that there is no exhaustion of the food supply by the bacterium which might be responsible for the destruction of the smut. It also shows that there is not a sufficient amount of enzymes or toxins present in the filtrate to affect the smut. However, Jones (9), in working upon enzymes of *Bacillus carotovorus* found that a large portion of the enzyme is adsorbed by the filter. He also found that a direct extraction of the enzyme with the proper reagents gives a much larger amount of it. Among such reagents, he used alcohol in sufficient quantities to make the broth culture 80 per cent alcoholic, then filtered it, and dried it quickly. This method was used in the present investigation of enzymes produced by this bacterium. Broth cultures of the organism were centrifuged, the bacteria removed with a small amount of the broth, shaken with sterilized glass beads until most of the bacteria were crushed, and finally extracted with alcohol, and filtered. Checks were run on the technique by making similar extractions from *B. carotovorus*. The residues from both cultures were tested for pectinase on slices of surface-sterilized carrot. Both broke down the cells of the carrot, reducing it to a soft mass resembling that of soft rot. The action of the residue of the crushed bacteria of Bamberg's culture was several times as rapid as that of the residue from the broth. Contamination was guarded against by the use of 1 per cent toluol. Inoculations were made from the carrot slices into broth to determine if any contamination might have occurred which could be responsible for the breaking down of carrot tissue. Only a spore-bearing bacterium was found, and this had no effect upon carrot. This also showed that Bamberg's culture had been killed by the alcohol and could not have produced the softening of the tissue. A further test on this point was made by inoculation of carrot slices with the or-

ganism, but it had no effect on them. Apparently, there is something present in the alcoholic extract of the bacterium which breaks down pectic material, but the organism itself is unable to attack carrot and produce soft rot in it.

It was difficult to obtain any definite and conclusive results in regard to the effect of the same type of residue on smuts in hanging-drop preparations. Jones states (9) that the residue of *Bacillus carotovorus* consists of precipitated proteins, bacterial cells, and the precipitated enzymes. That is probably true of the alcoholic precipitate from any bacterial culture. Toluol was added to the hanging drop to prevent growth of the sporidia, but it is not readily miscible with water, and growth of the sporidia occurred in patches in the drop. There was evidently, then, a constant increase of the smut. The same difficulty was encountered when chloroform was used instead of toluol. Under these circumstances, it would be difficult to determine the actual effect of the enzymes on the living, growing sporidia.

The determination of enzymes by cultural methods. A. Pectinase. The following medium was found satisfactory for this test: Magnesium sulphate, 0.25 gm.; dibasic potassium phosphate, 0.25 gm.; agar, 15 gm.; distilled water, 1,000 cc. To this was added 1 per cent pectin. Both a slightly acid and a neutral reaction were used. The bacterium grew well at both reactions, coloring the medium brown. No nitrogen was supplied, so the organism evidently obtained enough of it from the impurities in the agar, although it does not grow on agar alone nor on the medium without the pectin. The pectin used was from lemon, and the reaction of the medium was adjusted with calcium carbonate and sodium hydroxide. It seemed possible that some citric salts may have been formed from an impurity in the pectin and that these might have supported the growth obtained. The bacteria failed to grow, however, when the pectin was replaced by calcium and sodium citrates; apparently, therefore, it is the pectin that supports growth.

B. Chitinase. In testing for chitinase Benecke's (3, p. 241) medium was used. This consists of agar, 20 gm.; distilled water, 1,000 cc.; magnesium sulphate, 0.35 gm.; dibasic potassium phosphate, 0.15 gm. To this were added pieces of sterilized chitin. It is best to add the chitin after the agar is cooled. If added before, it sinks to the bottom. It was prepared from hard-shell crab, according to Benecke's (3) method, as follows: After removal, the exo-skeleton was soaked in dilute hydrochloric acid for a few hours to remove all adhering particles of flesh. Then it was boiled in 20 per cent sodium hydroxide for about 8 hours and washed until the reaction of the washing water was neutral. It was finally washed in alcohol and ether. Commercially prepared chitin also was used. This gave an acid reaction, so some of it was used without adjusting the reaction, while in

other portions the reaction was brought to neutral. Bamberg's culture did not grow on any of these media. Evidently there is no chitinase produced by this bacterium.

C. Cellulase. The bacteria did not grow upon filter-paper or upon precipitated cellulose. Cellulase evidently is not present.

MORPHOLOGY OF BACTERIUM B–1

The bacterium is a gram-negative, nonmotile coccus. It passes through a coarse filter but not through the finer ones. In filtering, checks were made by mixing the culture with yellow chromogenic bacteria and with *Bacillus carotovorus,* but these were never found developing in the filtrate. The number of individual cocci going through the filter is very small, for the filtrate is always clear, and no growth is evident in it for 4 or 5 days. In an active culture the growth is slimy, each cell being surrounded by a capsule-like exudate. A capsule cannot be demonstrated by the usual staining technique, but a dilute solution of methylene blue (1–10,000) stains only the central core, leaving a halo around the individual bacterium. This pseudocapsule stains with the usual bacterial stains, therefore the organism appears larger than it really is. It is extremely variable in size, depending on the type of medium upon which it is cultivated. When stained with the usual bacterial stains, it ranges from 0.7 micron to 0.9 micron. The central core is about 0.4 micron. Old cultures are dry and powdery, resembling precipitated chalk. The cells often occur in chains of four or five individuals, and one cell is usually larger than the others in the chain. In extremely old cultures these large cells are very numerous, and chain formations no longer exist. This suggests contamination, but transfers to fresh media do not give rise to a contaminated growth. It probably indicates a certain amount of pleomorphism.

This bacterium is apparently very prevalent. It was obtained several times from contaminated oat-smut cultures supplied by C. S. Holton of the Department of Plant Pathology. It also was isolated many times by the writer from discolored areas on corn plants at the point of inoculation with corn smut. It apparently is specific for smut. It has no effect on species of Fusarium, Verticillium, Penicillium, Aspergillus, and many other types of fungi not identified.

CULTURAL CHARACTERISTICS OF BACTERIUM B–1

The bacterium grows readily on most of the media. It is always extremely viscous and grows anaërobically and aërobically. It produces gas and acid from dextrose and mannite. On sucrose it produces gas, but no acid; on lactose, acid and no gas. On litmus milk it grows with an acid reaction, the milk finally being peptonized. It does not liquefy gelatin and has no diastatic action. There is nothing distinctive about its growth

on potato plugs. As previously mentioned, it grows on pectin, with a brown discoloration of the medium. It reduces nitrates to nitrites with a pronounced reaction in the sulphanilic acid-alpha-naphthylamine test used, as recommended by the Society of American Bacteriologists.

DISCUSSION OF THE ANTIBIOSIS OF BACTERIUM B–1

It would be difficult to decide just what factor is responsible for the destruction of the smut by bacterium B–1. The fact that the bacterium produces pectinase and the possibility of the presence of pectin in the cell wall of smut sporidia, especially in young sporidia, suggest that the cell wall may be dissolved. This is somewhat corroborated by the fact that débris and oil-like globules are present in hanging drops of a suspension of the sporidia and the bacteria. The destructive action of the bacterium on the young growth and not on the old also suggests some activity of the pectinase. As mentioned in the foregoing discussion, the old sporidial growth contains a preponderance of chitin and a substance staining blue with dilute methylene blue, which possibly may be cellulose or some closely related substance. Since neither cellulase nor chitinase was found in the organism, it is not likely that it produces any enzyme that could act on these substances. However, it is evident that the presence of pectinase alone does not account for its antibiotic action. Other bacteria, such as *Bacillus carotovorus* and *B. mesentericus,* have no destructive effect whatever on corn smut. On the other hand, Bamberg's bacterium does not produce a soft rot of carrot. Jones (9) concluded that in soft rot there was involved an "active osmotic substance." Evidently, each of these two organisms has its own specific antibiotic property. Possibly the destructive action of the bacterium in question cannot be ascribed to any one factor but may be the result of the interaction of a group of factors. Frost (6), in 1904, made a study of the antagonism of certan soil saprophytes to *B. typhosus.* His conclusions are probably applicable also to the results obtained by the writer on Bacterium B–1. They are, in part, as follows: 1. "The antagonism results in not checking the growth, but in actually killing the typhoid germs. In many cases the killing amounts to extinction." 2. "There is no evidence to show that the antagonistic substances exist ready-formed in the soil, but rather that the antagonism depends on the rapid development of the germs in the immediate presence of *B. typhosus.*" 3. "Changes in the environment of these organisms, such as temperature, oxygen supply, reaction of medium, amount of dextrose, etc., seem to have little or no influence on the production of the antagonistic substances. In other words, whenever the environment is such that a good growth of the organism occurs, the antagonistic substances are always produced." 4. "The cause of the antagonism is not due in the cases studied to the exhaustion of food supply, the action of

proteolytic enzymes, specific poisons, or the production of the hydroxyl ions supply."

Bamberg thinks that the organism B–1 may be of some economic importance, inhibiting the smut infection of corn plants to some extent under natural conditions. Whether it be of economic value or not, a study of it gives interesting information in regard to processes of antibiosis that are probably going on in nature.

OTHER BACTERIA ANTIBIOTIC TO SMUTS

In the course of some work on the black-chaff bacteria, the writer found several other types of bacteria that are antibiotic to smuts. As previously mentioned, these are designated by their laboratory numbers as bacterium C–1, D, and Myxobacterium–1.

Bacterium C–1

Bacterium C–1 is a small gram-negative rod. As far as it was studied, it is identical with Bacterium B–1 in its physiological reactions. It has a pseudocapsule, readily seen when dilute stains are used. It is constant in size; including the pseudocapsule, it is about 0.8 x 1.2 microns. The central core is about 0.5 x 0.8 micron. The growth is slimy, but it does not have the high degree of viscosity found in bacterium B–1. It grows upon the pectin media used for B–1. Alcoholic extracts were made similar to those made with B–1, but they had very little effect on carrot slices. The bacterium reduces large colonies of corn smut and of the oat smuts to a slimy mass with complete destruction of the sporidia. No other smuts were used in combination with it. Like Bamberg's culture B–1, its virulence is reduced by long-continued cultivation upon artificial media. When water suspensions of cultures of known virulence are inoculated into corn-smut galls, the latter are broken down and the young galls often turn green and disappear during growth of the plant. Like Bamberg's bacterium B–1, virulent cultures of C–1, when used in high concentrations in combination with sporidial suspensions, usually prevent gall formation in corn plants. It has no antibiotic effect on species of Fusarium, Penicillium, Aspergillus, Verticillium, and other soil fungi of unknown identity. It does not prevent the growth of fungi on the surface of soil moistened with broth cultures of it. It is, therefore, evidently specific for smuts.

Bacterium D

Bacterium D was found frequently as a contamination on agar plates by the writer in the course of some isolations of black-chaff bacteria. Contaminating colonies of corn smut and this bacterium were often present on the plates, and wherever the colonies of the bacterium came in contact with the smut colonies the bacteria overran the smut colonies and finally de-

stroyed them. Scores of smut cultures were inoculated with this bacterium and always with the same destructive effect. In the course of 10 to 14 days the sporidia in smut colonies an inch or more across were completely replaced by the bacteria. The colony retained its original shape and contour, the only change in the appearance of the smut growth being the slight discoloration as the bacteria advanced. Figure 1 shows a smut colony so

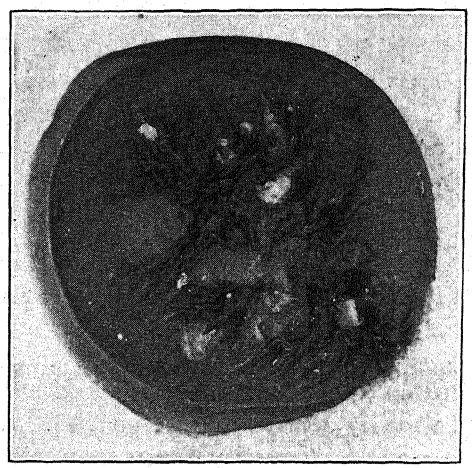


FIG. 1. Colony of *Ustilago zeae*, inoculated with bacterium D.

replaced by the bacteria, and Figure 2, A, shows a stained mount of the colony at this stage. For comparison a mount of only sporidial material at the same magnification is shown (Fig. 2, B). Like the previously discussed cultures, bacterium D does not affect the old smut growth. This is not because of the dryness of the smut culture, since the addition of sterile water at the time of inoculation did not increase its antibiotic effect.

Inoculations were made into young corn plants with a water suspension of sporidia mixed with a water suspension of the bacterium. Only one of three dozen plants developed galls, while 100 per cent infection occurred in two dozen plants, inoculated with sporidia only. The infection in that one plant may have been due to a survival of one pair of the proper strains of sporidia rather than to the inactivity of the bacterium. Another set of plants was inoculated to check this experiment, but no conclusions could be drawn from them, because no infection occurred in the checks.

Attempts also were made in the greenhouse at temperatures ranging from 14° to 20° C. to determine the effect of the bacterium upon the infection of wheat and oats by the various smuts. Inoculations were made both with cultures and with chlamydospores of the smuts and the soil was kept moist with bacterial cultures. Results were inconclusive, since no smut

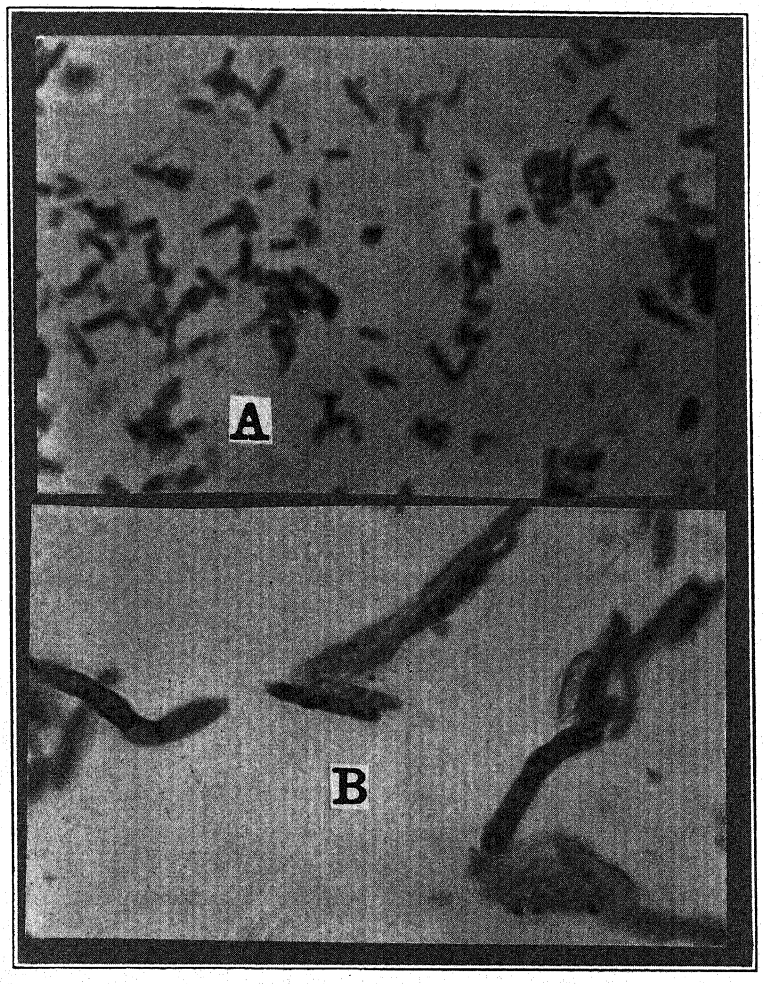


FIG. 2. A. Stained mount from the colony of *Ustilago zeae* shown in figure 1. The sporidia have been replaced by the bacteria. (×1,200.) B. Stained mount from sporidial material. (×1,200.)

developed in the checks. Rodents and birds repeatedly destroyed the plantings outside, so no idea was obtained as to the rôle of the organism in the infection of wheat and oats by smuts.

During some inoculation experiments to determine whether the organism can prevent bunt and oat smuts from infecting the seedlings in the soil, there was noticed the complete absence of Pyronema and other fungi from the tops of the pots kept moist with the broth cultures of the bacterium. Without exception, the checks, to which only sterile broth was added, were

covered with fungus growth. To check further on this observation, a series of pots of soil was used in another experiment in which one set was kept moist with a broth culture of the bacteria, another set being moistened with broth without the bacterium. The former remained free from fungus growth, while the latter was covered with it. In another set of pots inoculations were made with various fungi found growing on soil; some were moistened with a broth culture of the bacterium and some with broth only. The results were the same as in the previous series. Figure 3 shows a pot

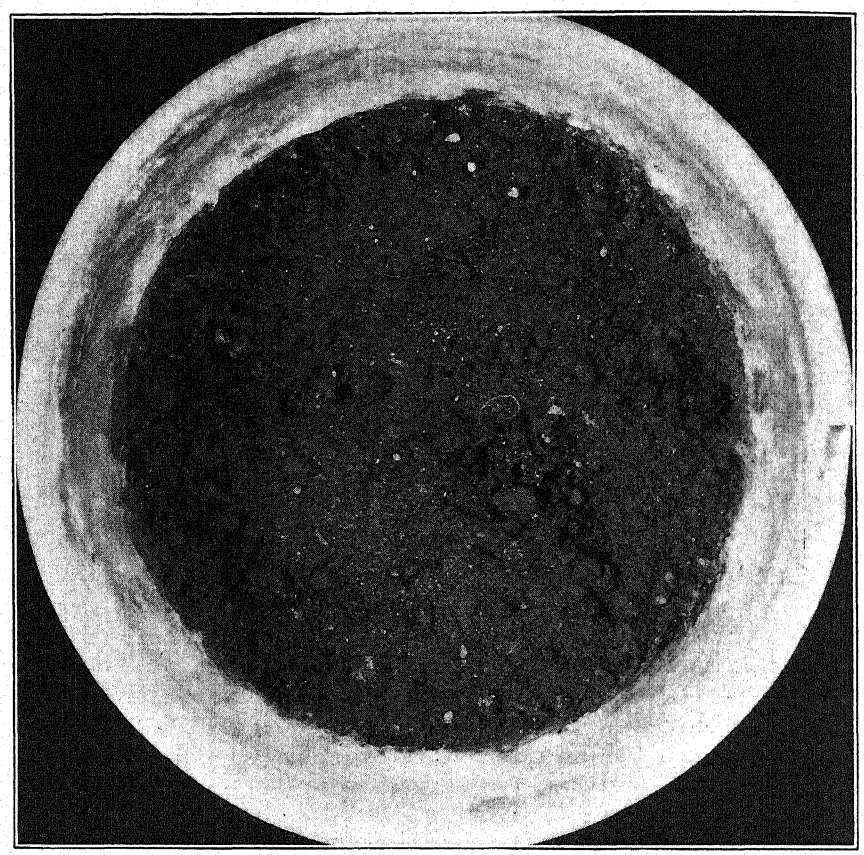


FIG. 3. Soil inoculated with a mixture of soil fungi, moistened with a broth culture of bacterium D, and showing the absence of fungus growth.

so inoculated with fungi and moistened with the broth culture of the bacteria. The surface shows no fungus growth. Figure 4 shows a check, a pot inoculated with fungi and moistened with broth alone. It is overgrown with Pyronema and Penicillium. Evidently, bacterium D is active in the soil and has an antibiotic effect upon some soil fungi.



FIG. 4. Check: Soil inoculated with approximately the same amount of soil fungi, moistened with growth without bacterium D, and showing growth of Pyronema and other soil fungi. Compare with figure 3.

No enzymes of any kind could be demonstrated in bacterium D either by cultural methods or by precipitation of broth cultures with alcohol.

Specificity. A. In artificial media. The organism destroys colonies of *Ustilago zeae, U. levis,* and *U. avenae.* It was not tried on other smuts. It also kills one species of Penicillium. It remains in a vegetative condition for a long time in liquid cultures of other species of Penicillium but is finally crowded out. This may be due to a difference in rate of growth. It does not affect species of *Verticillium* and *Fusarium.*

B. In soil. As previously stated, no conclusions could be drawn from the inoculations of the smut and the bacterium into the soil because no smut occurred on the checks. However, as shown, the growth of many types of fungi was inhibited. Possibly the high buffer content of the soil protects the bacterium against excessive acid. In a liquid medium excessive

acid is unfavorable to the growth of the bacterium and produces sporulation.

Cultural characteristics. The bacterium produces no gas. It produces acid on dextrose but not on lactose, sucrose, or mannite. It has a proteolytic action on milk and liquefies gelatin rapidly. It does not produce indol. It has no distinctly characteristic growth on potato and does not produce diastase. It is not chromogenic. Under certain conditions it grows in a star-shape colony. It probably belongs to a group of spore bacteria with this same characteristic, for two other cultures were isolated with this same type of colony but they differed from this bacterium in their sugar reactions and had no destructive effect on smut.

Morphological characteristics. The bacterium is a motile spore bearer. It is variable in size, depending on the richness of the medium, ranging from 0.6 x 2 to 1 x 3 microns. On media rich in dextrose and protein it assumes very bizarre shapes. It is somewhat pleomorphic, the rod-shape forms often breaking up into round bodies which stain with the common bacterial stains. The cells usually occur singly, but short chains may be found. It is gram-positive in young cultures, but the cells gradually lose this property in old cultures.

Discussion of the antibiosis of bacterium D. On sugar media the bacterium sporulates quickly, but, if smut is added to a broth culture reduced almost completely to spores, the culture starts growing and may continue in the vegetative form for weeks. The same is true when inoculations are made with certain species of Penicillium and other fungi. It may be possible that the fungi utilize acids and other metabolic substances that would otherwise cause staling of the bacterium. Hill (8) found that certain species of Penicillium activated cultures of certain luminous bacteria. When filter-paper medium was inoculated with the bacterium D no growth occurred. Finally the medium became contaminated with a Penicillium, which grew upon the inorganic salts solution and spread over the filter-paper without affecting the paper. Wherever the Penicillium grew there was abundant growth of the bacterium, and it continued to exist in the vegetative form. The bacterium destroys the sporidia in smut colonies an inch or more in diameter in 10 to 14 days. The smut colony retains its shape and general characteristics, the only indication of the advance of the bacterium being a slight change in color of the smut colony. The bacteria replacing the smut culture are in the vegetative form as long as there is smut left. The bacteria on the agar medium surrounding the smut are in the spore condition after a few day's growth. In the broth culture the antibiotic effect on the smut is not so pronounced; the bacterium prefers aërobic conditions, and the corn smut grows well under semiaërobic conditions. This may hamper the bacterium to some extent. Then, too, the

bacterium is sensitive to acid. The agar, with its high buffer content, probably protects it against this staling product, enabling it to continue its growth upon the smut, while the broth does not contain so much buffer material. The inhibition of fungus growth upon pots of soil inoculated with fungi and moistened with the bacterial culture suggests the same thing, that the buffers in the soil probably maintain a favorable reaction for the bacterium. It seems evident that, when other conditions are suitable, the bacterium obtains some element of food from the fungi to which it is antibiotic.

It is difficult to suggest what the antibiotic substance is. Sporulating cultures that had been boiled vigorously for 10 minutes, then allowed to germinate, did not lose the antibiotic property. As stated before, no enzymes were demonstrated that could dissolve the cell walls of the sporidia. It produces no acid which might be destructive to them, for the filtrate of the bacterial culture supported growth of the smut. Its virulence is destroyed by constant cultivation upon artificial media. With all findings negative, it would seem again that the antibiotic action may be due to a factor or a group of factors that are not readily demonstrated.

Myxobacterium–1

A Myxobacterium, also, was found by the writer in the course of some work on black chaff. It appeared as a pink streak in a corn-smut colony contaminating an agar plate. At first it was thought to be a Fusarium and was kept under observation to see which one would crowd out the other. Further study showed that it consisted of rod-shape bacteria and round spores, the latter forming globular or somewhat oval fruiting bodies. This development corresponds to that of the Myxococcus, a genus of the Myxobacteria.

According to Quehl (16) and several other investigators, Link described a Myxobacterium in 1795, but he thought it was a Gasteromycete. Schroeter recognized the bacterial nature of the organism and described two species in 1889. However, he made no further study of them. Thaxter (18, 19, 20, 21) made the first extensive study of the group. This work was followed by a series of articles by European workers, among whom are Baur (2), Kofler (10), Quehl (16), and Vahle (23). More recent work has been done by Helena Krzmieniewska and S. Krzmieniewski (11, 12, 13) of Poland. Emoto (4) of Japan recently has published a list of the investigations on the group up to date.

Faull (5) has summarized the characteristics of the myxobacteria as follows: "The Myxobacteriaceae constitute an extremely interesting assemblage of forms because of their apparent relationship to the bacteria on one hand, and to the slime molds, particularly the Acrasieae, on the other. The

individual plants are bacteria-like rods in all cases, and after a vegetative period, these swarm together to organize a definitely shaped pseudo-fructification, without, except in Myxococcus, undergoing any marked morphological changes themselves. These fructifications are not comparable to the more or less heaped-up colonies characteristic of certain bacteria; but, as Thaxter has pointed out, are strictly comparable to the fructifications of the Acrasieae."

Myxobacteria have been isolated from many sources, such a dung, rotting wood, decaying leaves, lichens, fungi, and filter-paper, and once from a bird's nest. Thaxter (18) in 1892 described one of them, a species of *Chondromyces,* as "parasitic upon living lichens, which it destroys." Geitler (7) in 1924 isolated a species of Polyangium, which invades the cells of a Cladophora species and destroys this alga.

Myxobacterium–1. As previously mentioned, this culture was first observed growing on a smut colony contaminating an agar plate and appeared as a pink streak in the smut growth. It evidently belongs to the genus Myxococcus. It consists of a rod-like, often slightly curved, vegetative form, and in its later growth produces round spore-like bodies which form globular or slightly oval fruiting bodies. Among the species of Myxococcus described, it resembles *Myxococcus rubescens* Th. more than any other. However, Vahle (23) and, later, Yoshi (25) have reported that *M. rubescens* liquefies agar. Since liquefaction has not been obserevd in the case of Myxobacterium–1, it does not correspond entirely to the description of *M. rubescens.* So far, the work on the myxobacteria deals with the source, method of isolation, and the morphological characteristics of the group. Until a comparative study, including the physiological characteristics, can be made, it would not be possible to identify the species discussed here. So much work has been done on the morphology of the group, the most recent being on those species found in Poland, that no general discussion is necessary.

Myxobacterium–1 is colorless in its vegetative stage, but, when the pseudoplasmodium begins to draw together to form fruiting bodies, the colony often acquires a pink tinge. The fruits range from a faint to a dark pink. Several times they have turned to a brick-red, when growing on old smut growth. Fruiting bodies are formed much more rapidly on drying media, although they finally form even in a liquid medium. Preliminary to fruiting the vegetative mass evidently seeks the high, dry places, wherever this is possible, for the fruits are usually found on elevated areas. Figure 5 shows fructifications on the ridges and protuberances of old smut growth. On chitin the fruits almost always appear at the apices of the pieces. Figure 6, A, shows the lateral view of the characteristic position on chitin.

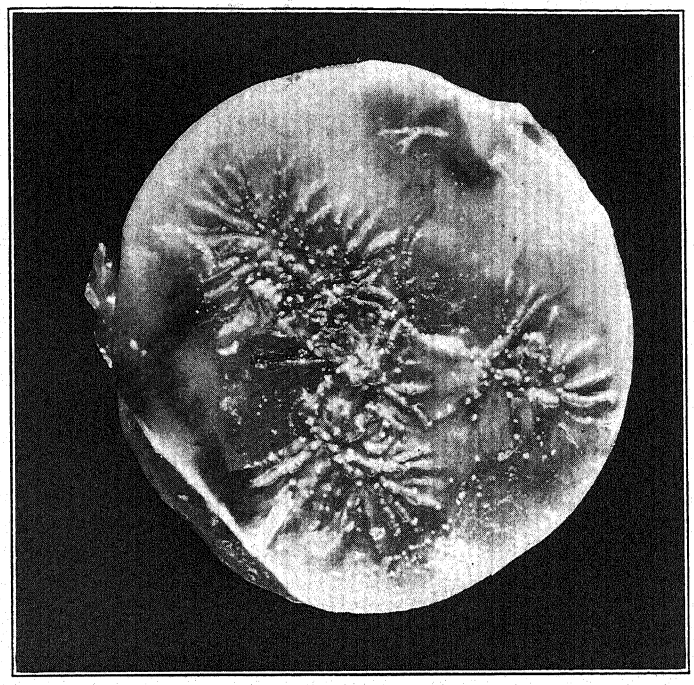


FIG. 5. Large colony of *Ustilago levis* with the fruiting bodies of Myxobacterium-1. (Natural size.)

Cultural characteristics. The best medium for this Myxobacterium is young, vigorously growing corn smut or the oat smuts. The smut becomes covered and permeated with the colorless vegetative growth of the organism. In about 10 days fruiting occurs, the smut colony being covered with pink, bead-like fruits, placed on the elevations of the smut. These may remain intact for several months. The bacterium grows well on Kofler's cane-sugar medium of the following composition: Peptone, 2.5 gm.; cane sugar, 15 gm.; magnesium sulphate, 0.25 gm.; agar, 9 gm.; tap water, 500 cc. It also grows well in a liquid medium of the same salts and organic constituents. The following precipitated cellulose medium is very favorable: Ammonium magnesium phosphate, 2.0 gm.; dibasic potassium phosphate, 0.25 gm.; magnesium sulphate, 0.25 gm.; sodium chloride, 0.1 gm.; ferric chloride, trace; agar, 15 gm.; precipitated cellulose; tap water, 1,000 cc. This gives a slightly alkaline reaction, which seems favorable to the organism. At times a very scant growth has been observed on the medium without the precipitated cellulose, but the difference in the amount of growth is so marked that it can undoubtedly be assumed that the precipitated cellulose serves as a food element. It also grows readily on filter-

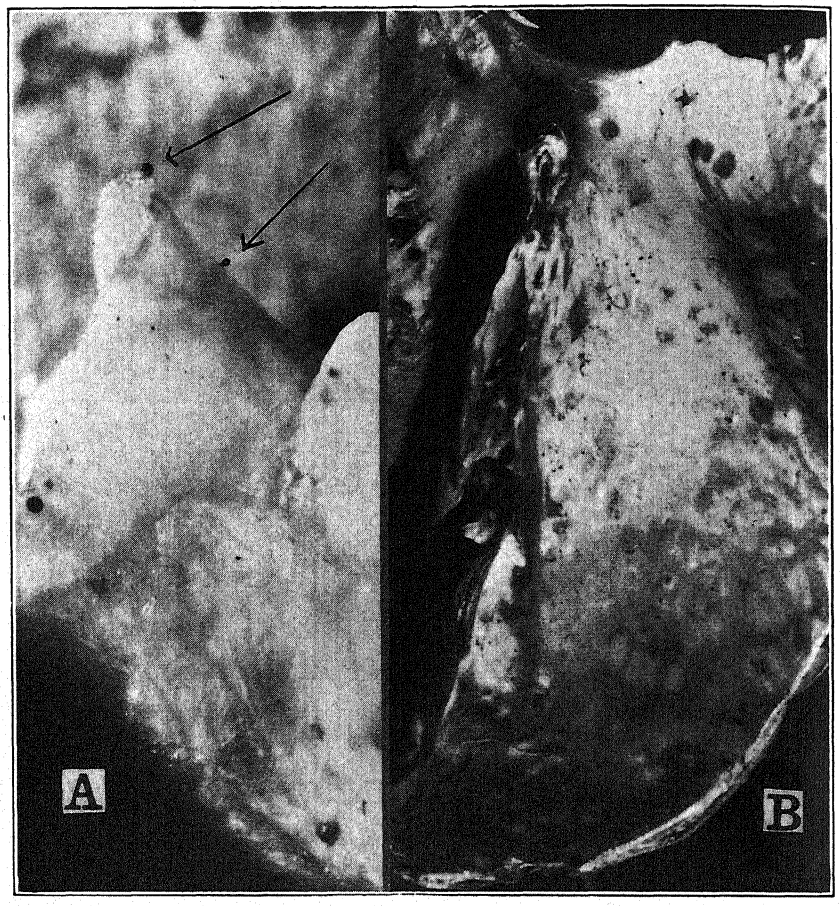


FIG. 6. A. Chitin pieces showing fruiting bodies of Myxobacterium-1 in characteristic position on the higher portions of the medium. (×6.) B. Fruiting bodies of Myxobacterium-1 scattered over a piece of chitin. The chitin was originally white, but it has been darkened by the bacterial growth. (×6.)

paper medium, consisting of the salts used in the precipitated cellulose medium, 1 per cent agar, and shredded filter-paper of a good grade. Shredding is accomplished by the use of a nutmeg grater. The paper absorbs so much of the water in the medium that it becomes too dry to support growth if more agar is added. It does not grow on filter-paper of the same grade when this is suspended in a liquid solution of the same salts. If the suspended filter-paper is inoculated with both the bacterium and the corn-smut sporidia, the latter grow fairly well on the liquid medium and the colonies spread over the moist filter-paper. These smut colonies usually are centrally pink, as a result of the growth of the Myxobacterium on the smut.

The organism also grows fairly well upon the pectin medium that was used in culturing bacterium B–1 and C–1, turning the medium a dark brown. It grows upon the chitin medium previously described, turning the chitin a dirty pink and gradually softening it. Figure 6, B, shows the fruiting bodies scattered over a piece of chitin. The chitin was originally white and has been blackened by the bacterial growth. Sterile water must be supplied often enough to keep the chitin moist or no growth takes place. Growth on chitin is slow, several months being required before any perceptible change occurs. No further study of the food requirements of the bacterium was made.

Effect on smut growth. When the bacterium is inoculated into young corn-smut cultures, the fructifications occur, as previously mentioned, on ridges and elevated portions of the smut. Examination with a hand lens shows that the protuberances are levelled down, so the part overrun by the bacterium has a smooth surface, while the areas that are not overgrown have a rough surface. The bacteria confine themselves to the smut, never spreading beyond it upon the media.

Specificity. The organism grew upon corn smut and the oat smuts. It was crowded out by Penicillium, although fruiting bodies of Myxobacterium frequently were found among the hyphae of Penicillium. No further study was made of its specificity nor was its economic importance determined.

Discussion of the antibiosis of Myxobacterium–1

Myxobacterium–1 has not been found within the smut cells, so its action must be extracellular only. It evidently is able to produce chitinase, pectinase, and cellulase. If it be a fact that the cell walls of the corn and oat-smut sporidia contain chitin, pectin, and cellulase, or some closely related substance, as microchemical tests suggest, then this bacterium may be able to attack and dissolve the cell wall of the sporidia. However, as previously mentioned, not all bacteria producing pectinase are antibiotic to smuts. Similarly, not all bacteria that produce chitinase have a destructive effect on the smuts studied. For example, another species of Myxococcus and an Actinomycete were found which break down chitin but which have no effect whatever on smut. Evidently, then, the presence of the enzyme is not the determining factor. If it play a part in the antibiosis, there must still be some other factor involved which has not been demonstrated in the course of this investigation. Probably the outstanding result of this study is the evidence obtained that several groups of bacteria are concerned in such antibiotic processes going on in nature.

SUMMARY

1. Four types of bacteria are discussed which are antibiotic to certain smuts and other fungi. They consist of a coccus; a motile, nonspore-bear-

ing, rod-like bacterium; a motile, spore-bearing, rod-like bacterium, and a Myxobacterium.

2. A study of the enzymes of these bacteria was made to determine if they can break down the cell wall of the sporidia and so destroy the smut growth.

3. Sporidia of corn smut were stained to determine the chemical constituents of the cell wall.

4. Some of the bacteria contain enzymes with which they may be able to dissolve the corresponding chemical constituents of the cell walls of sporidia.

5. However, certain other bacteria, with the same types of enzymes, do not affect the sporidia of the same smuts. There are, therefore, probably other factors involved in the antibiotic property.

6. The cultures become avirulent after long cultivation upon artificial media.

7. Some experimental work was done on all the cultures except the Myxobacterium to determine their effect upon infection of corn plants by smut. The results indicate that they may inhibit infection to some extent under proper conditions.

8. While it is impossible to make any statement as to the economic importance of this antibiosis, the study suggests that antibiotic processes occur in nature.

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YELLOW SPOT OF PINEAPPLES IN HAWAII

J. F. ILLINGWORTH[1]

INTRODUCTION

A destructive disease of pineapples, new to science, has demanded during the past 4 years the serious attention of the scientific staff of the Experiment Station of the Association of Hawaiian Pineapple Canners, in Hawaii. Observed first as a distinct disease, in the spring of 1926, narrow in its range of distribution, it has since become wide-spread and at times exceedingly destructive.

This paper is designed to present a picture of the nature of the disease, giving a history of its development, with observational and experimental data leading to the discovery of the probable vector, a species of thrips. Only tentative control measures are suggested. Further study of the problem is in progress by other workers.

Dealing with this disease, Dr. C. P. Sideris made the first studies.[2] During the next 2 years the disease assumed alarming proportions, and Mr. Glenn E. Paxton reported fully,[3] with excellent illustrations, summing up our knowledge of the subject to date, at that time. Both papers were published only in the local organ of the pineapple industry.

DESCRIPTION OF THE DISEASE

The first appearance of this disease, the so-called "initial spot," is a slightly raised yellowish spot on the upper surface of the leaf. It varies in size from $\frac{1}{8}$ to $\frac{1}{2}$ inch in diameter. When fully developed the darker center is surrounded with a halo of yellow (Fig. 8). Ordinarily only 1 leaf on a plant is thus affected, but in extreme cases we have found as many as 5 initial spots on a single plant. When the spot makes its appear-

[1] This study has not been an individual effort. It was carried on jointly by the Entomological and Pathological Departments of the Station. Mr. Glenn E. Paxton, of the latter Department, cooperated with the writer closely in all of the later field work. Furthermore, it was his suggestion in April, 1929, that side rot might be a virus disease, which started us on the right track to our goal. Dr. Royal N. Chapman, head of the Department of Economic Entomology at the University of Minnesota, also was associated with the investigation for a brief period, during October and November, 1929. He made a most important contribution to the investigation, discovering the characteristic, microscopic, insect punctures associated with the yellow spot on the leaves. Finally, the work of Dr. M. B. Linford, of the Pathological Department, indicated that the vector was an insect of very small size, which embedded its egg in the pineapple leaf. It was upon the basis of this work that the vector was suspected to be a species of thrips.

[2] Side rot of pineapple plants, March, 1927.

[3] "Side rot" or "yellow spot" disease, June, 1929.

ance it is 3 to 8 inches from the base of the leaf. This is due to continued basal growth during the fairly long incubation period. After infection takes place, in the axillary region of the leaf, 10 to 20 days must elapse before the yellow spot is apparent. The rate of growth of the individual plant determines the distance up on the leaf. All the evidence goes to show that the insect infection takes place near the center of the plant, for the initial spots appear on the leaves of the third or fourth whorls from the center.

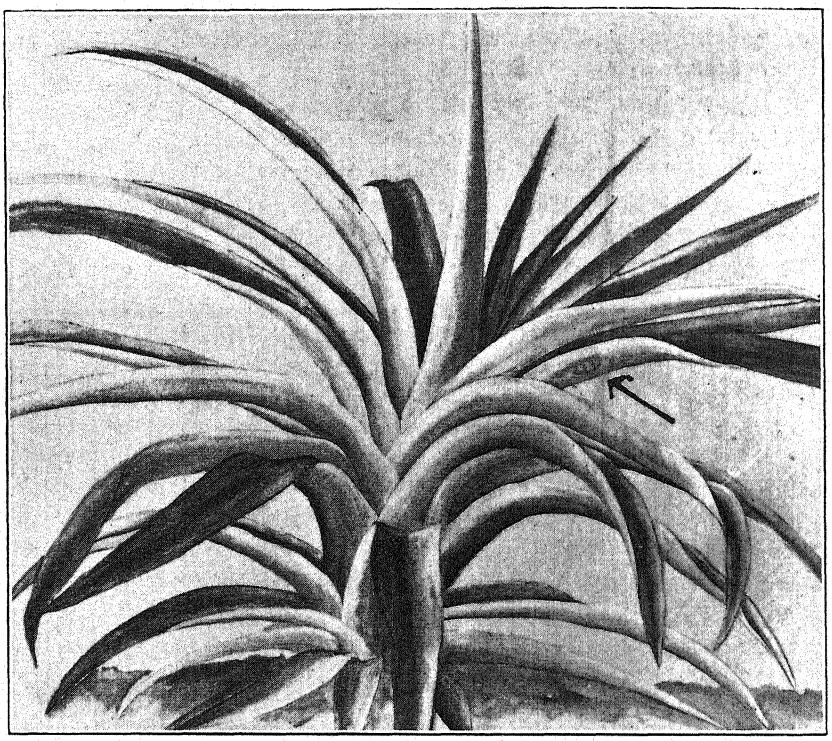


FIG. 1. Young pineapple plant suffering from an advanced stage of yellow spot. Note the characteristic tipping of the plant to the right. The arrow points to a spot on a leaf, where infection took place. Photograph of colored plate by Armena Eller.

Under favorable conditions, a yellow streak develops directly below the initial spot, widening in the region of the white tissue at the base of the leaf. The tendency of this streak is to become constricted into circular yellow blotches, giving it the appearance of a chain of beads. These usually start an inch or more below the initial spot. After a few days the portion of the streak in the white tissue, at the base of the leaf, has a water-soaked appearance. In the presence of moisture in the leaf axils, rot soon follows,

extending to the stem. A few days later, a yellow streak, developing into the characteristic bead-like chain, can be observed extending up the next leaf above the one first affected. This usually spreads quickly to the other central leaves, and the whole plant is doomed.

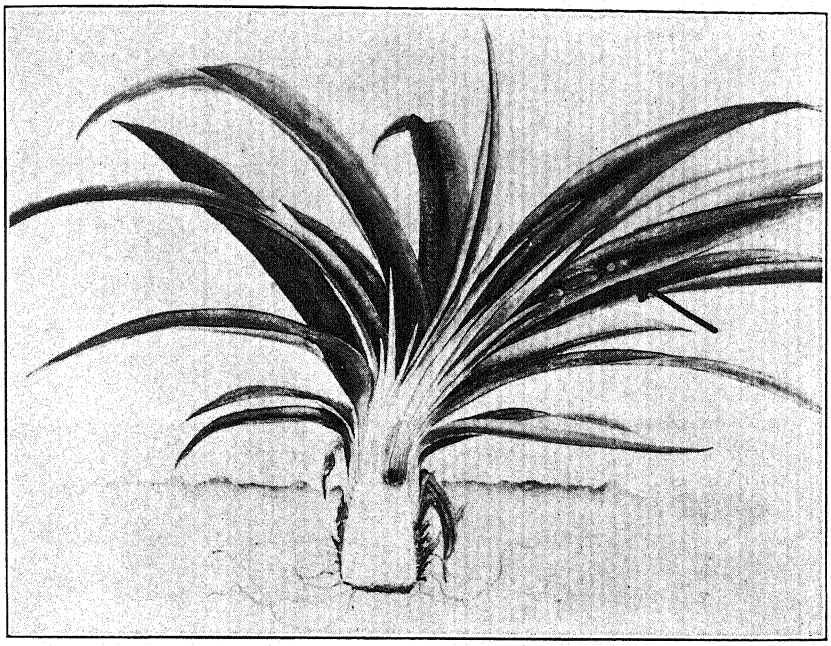


FIG. 2. A median section of the plant shown in figure 1. Note how the rot is progressing downward to the stem. The initial infection on the leaf is indicated by the arrow. Photograph of colored plate by Armena Eller.

At the point where the tissue of the stem is affected, the stem ceases to grow. The normal development of the healthy part, on the opposite side, soon causes the plant to bend over very decidedly (Figs. 1 and 2). This led at first to the name "side rot" to designate the trouble.

Side rot is primarily a disease of young plants. Of the three types of planting material, tops are by far the most susceptible because of their loose structure, permitting the vector easy access to the tender tissue in the leaf axils. Tops, while still attached to the fruit, may also be affected (Fig. 3). This usually follows in a field that has previously suffered from the disease on the plants. Yet, in several instances, it occurred first on the foliage of the fruits, in fields where the disease had not troubled the plants.

History of its development. This disease first came to my attention May 24, 1926. It appeared in a young field on Oahu, located in a fairly

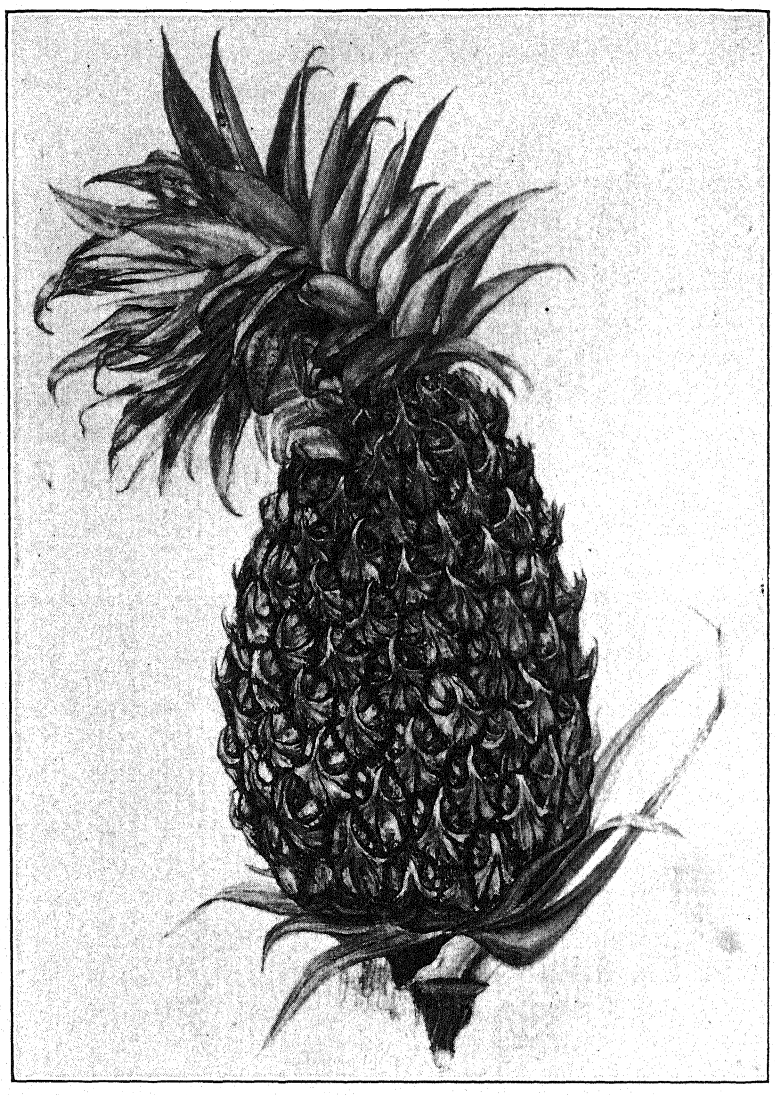


FIG. 3. An advanced stage of yellow spot, showing its effect upon the top. Here most of the leaves had died and rot was progressing downward into the fruit. Photograph of colored plate by Armena Eller.

rainy section. After the warm weather came on, in June, the trouble ceased. That was an exceptionally dry year. With cooler weather and occasional showers in December, the tops on the fruiting pineapples in this same locality began to suffer from the disease, resulting in the rotting of many of the fruits.

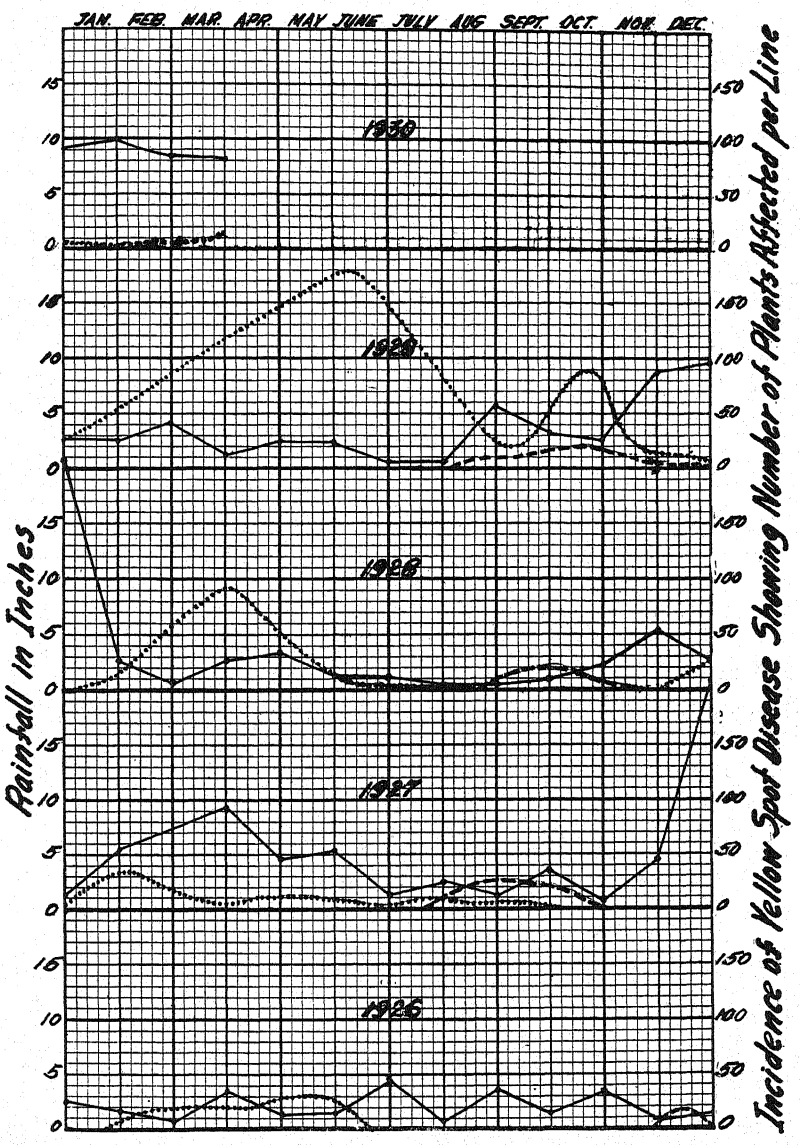


FIG. 4. History of yellow spot in relation to rainfall. Rainfall indicated by solid lines; virulence of disease on young plants, by dotted lines; on tops (attached to fruits), by dashes. The rainfall records are those of Schofield Barracks in the heart of the pineapple section on Oahu and are fairly representative of the fields from which the disease data are recorded.

The season of 1927 opened with increased rainfall (Fig. 4) and the disease made rather slow progress. A new outbreak, however, extended the range to a distance of several miles, into a fairly dry region. The disease also appeared on tops of fruits in a field planted with suckers. This indicated that we were dealing with a new primary infection.

The spring of 1928 was a period of few intermittent rains and the trouble increased materially over what it had been in the 2 previous years. It not only was more virulent in the original locations but continued to spread to more distant centers. However, the trouble ceased as hot, dry weather came on at the end of May. The disease began to appear again in December after the rainy season started. We were at a loss to know the cause of the trouble. Sick plants were pulled and carted out of the fields by dray loads.

The year 1929 opened with low rainfall, and the disease again developed with increasing virulence (Fig. 4). The spread was tenfold what it had been in previous years. It now appeared in practically every section where pineapples were grown on Oahu. Furthermore, it continued into the summer; in fact, it never completely disappeared, for diseased plants could be found throughout the hottest and driest weather. The trouble appeared to be slowed down only by these adverse conditions. It now became evident that the disease was systemic. In fields hit the previous year, we found all types of planting material while still attached to the parent plant, showing the characteristic yellow spots, with leaves rotting at the base. Tops, slips, and suckers were affected alike. Evidently the parent plant had carried the trouble over from the previous season.

In June, 1929, the disease was reported from the eastern end of Molokai, probably taken over on the planting material from Oahu. We made a careful survey and found conditions very similar to those on the island of Oahu.

In September, 1929, there was an exceptionally early outbreak of yellow spot in a young field, located near the original center of the trouble on Oahu. Virulence increased until rains set in, when the disease disappeared as suddenly as it came (Fig. 4). Here the weather seemed a very important factor. During November, 1929, this disease was found to be fairly widely distributed on Maui. This left only the islands of Kauai and Hawaii unaffected.

Observational and experimental data. As I have indicated, climatic conditions evidently play an important part in the activity of the disease. The relation to rainfall is clearly shown in the graph, figure 4. Even after the initial spot makes its appearance further progress of the disease is determined by weather conditions. Humidity hastens the disease, while drought slows it down for months.

Long before we knew what was causing the disease, we began to notice a very definite relation to the prevailing winds coming from old fields. A good illustration is that on east Molokai: in this case, the infected area was limited and appeared to have a very definite relation to the prevailing winds coming from old weedy fields in which tops were badly affected with the disease (Fig. 5). We could not find a trace of the trouble anywhere else on Molokai. Young fields lying not more than a quarter of a mile away from this infested center showed perfect growth.

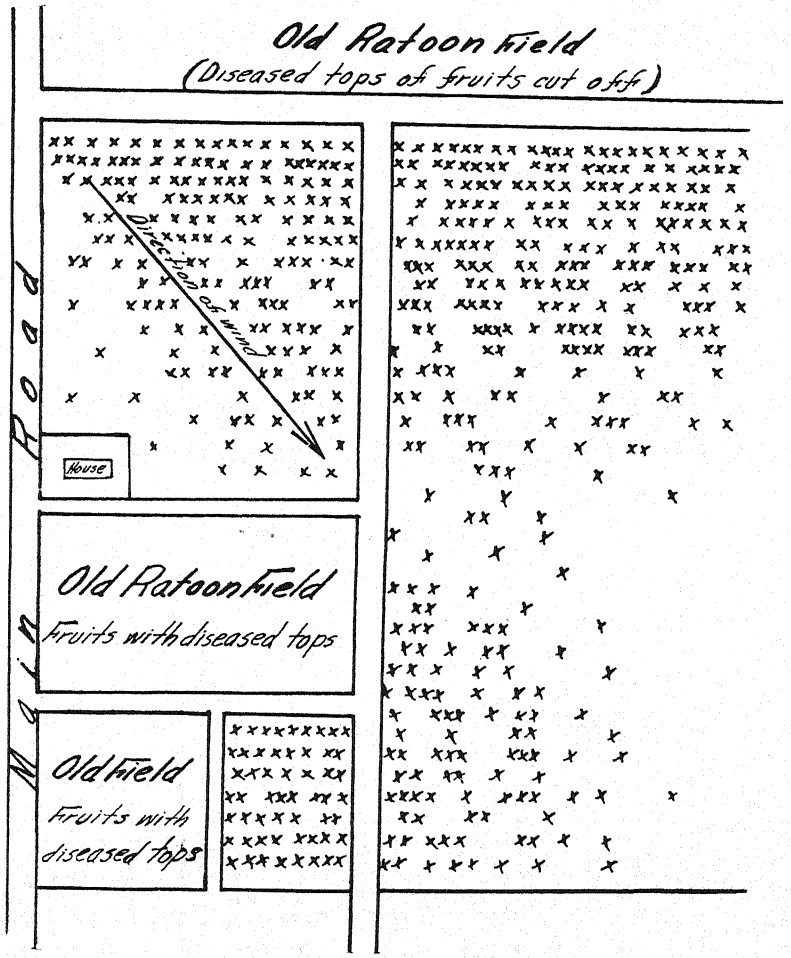


FIG. 5. Sketch to show the relation of infection of yellow spot to prevailing winds on Molokai. The old fields were very weedy, and most of the pineapple tops had been cut off, because they had been attacked by this trouble. Diseased plants are indicated by × marks (each "×" representing several plants).

This wind relation I had found in evidence in several places on Oahu. As a typical example I will cite a field of 77 acres at Kunia, which is a fairly arid section (Fig. 6). The windward area of this field suffered serious injury from yellow spot. Careful counts of plants demonstrated that the disease gradually diminished toward the leeward side, where there was no evidence of the trouble whatsoever.

Mr. Paxton and I began a study of field weeds early in April, 1929, following his suggestion that this might be a virus disease with some weed as a host. At that time we decided to call the trouble "yellow-spot" dis-

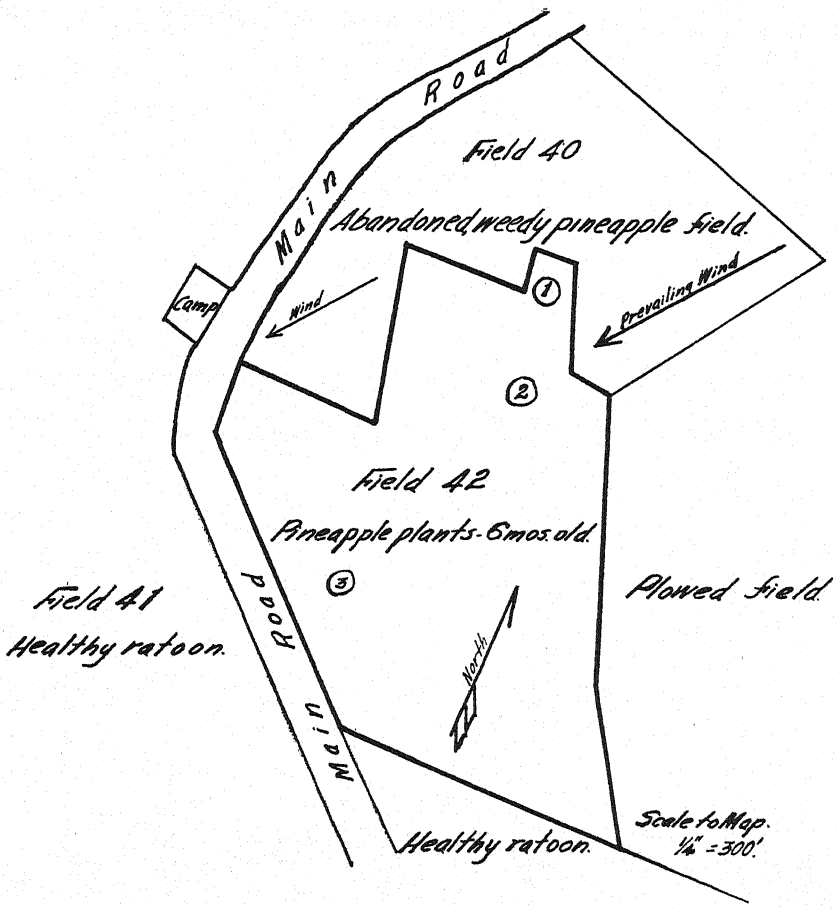


FIG. 6. A 77-acre field at Kunia on Oahu, which showed a decided wind infection, from an abandoned, weedy area adjoining. In the portion of the field marked 1, the loss from yellow spot was extremely heavy. A little further in, 2, the losses were not more than one-half as much, while at the leeward side, 3, there was no indication of the trouble.

ease, because of the confusion in the use of the name "side rot" with several other rots on pineapples.

We collected all the various insects found on weeds in the affected fields, to determine what vector was transmitting yellow spot. Preliminary trials were made with the following insects, enclosing them in large lantern chimneys over pineapple plants, both in the field and in our greenhouses: (1) Nysius bugs, from Erigeron, Portulaca, and Bidens; these bugs were observed frequently down in the heart of young pineapple plants, especially following weeding of the fields. (2) Plant lice, from Sonchus, nightshade, Portulaca, Emilia, and several other weeds. (3) Jassids, from nightshade and Paspalum grass. (4) Thrips, from Emilia flowers and leaves. Results from these preliminary trials were all negative, as were likewise our numerous attempts to reproduce the disease artificially.

Dealing with susceptibility to yellow spot, I critically studied the various types of planting material, especially as to structure and its effect upon insect population.[4] Even a casual observation indicated that tops, due to

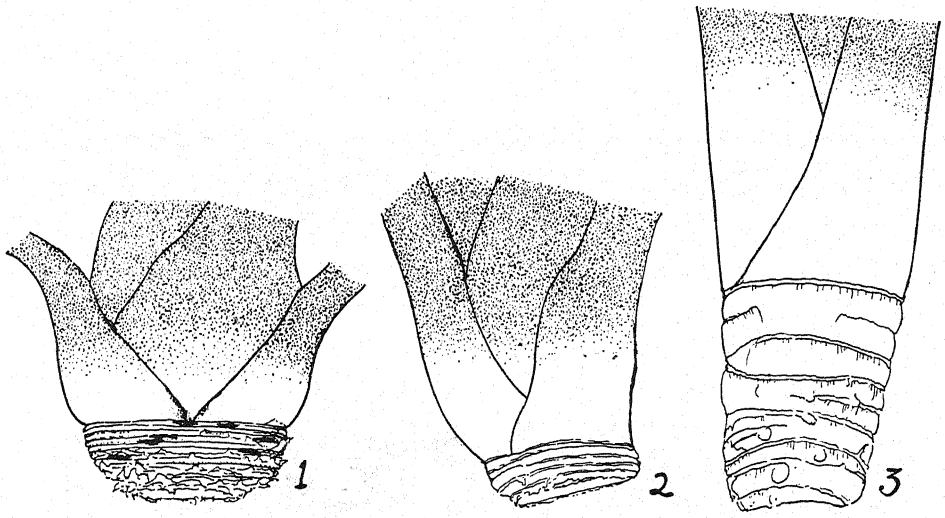


FIG. 7. Sketches of the butts of stripped Cayenne planting material. 1. A crown showing leaves loosely attached and not coming together at edges. Here we see discoloration, where insects, mites, etc., are usually secreted, feeding on the very tender white tissue. The dark spots on the stem are the early stages of injury by the fungus Thielaviopsis, initiated by these pests. 2. A slip showing overlapping of the leaves at their edges. Due to the fact that they clasp the stem more tightly, the white area at the base is considerably greater than seen in the crown. 3. A sucker, showing extremely tight imbrication of the leaves about the stem. It is practically impossible for thrips to infect this type of plant. (Engraving, courtesy of Experiment Station, Association of Hawaiian Pineapple Canners.)

[4] Leaf characters and resistance to yellow spot. Reported on in Station files for October, 1929.

their open, spreading leaves, have a much greater insect population than suckers (Fig. 7). The closer imbrication of the leaves in the latter evidently accounts for their relative immunity from yellow spot.

During the period July to September, 1929, further experiments were carried on with possible vectors. Thirteen kinds of insects were used. Besides those studied previously, all of the following various organisms found in my study of structure of pineapple planting material were now included: Tarsonemus mites, red spider, and a species of thrips that rather constantly occurs with them, pineapple mealy bugs, spring tails, bud-moth caterpillars, Chironomid maggots, Nitidulid beetles, and *Scolia manilae* wasps. All of these are closely associated with the growing pineapples, but none of them gave positive results.

The quest for the vector was now taken up on a much-increased scale. Plant lice were again studied thoroughly. Jassids of two species, rather abundant on weeds in the affected field, were caged by hundreds with young pineapple plants. They appeared to feed normally on the tender tissue at the base of the leaves and even inserted their eggs in crescent-like slits that they cut in the epidermis. On October 23, I found a pineapple leaf in the field with similar punctures containing fresh eggs. From none of these studies, however, was yellow spot reproduced.

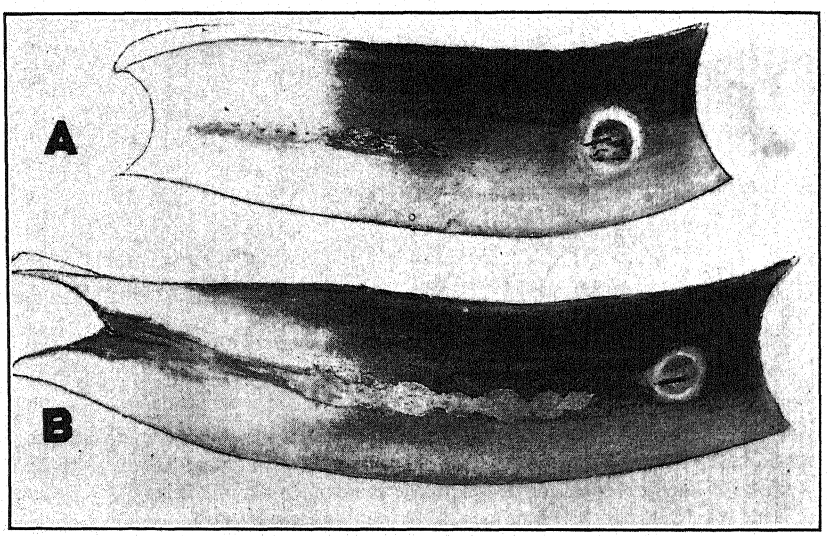


FIG. 8. Two pineapple leaves showing initial yellow spots above. A. An early stage of the disease, with the water-soaked areas just beginning to appear at the base, indicated by shading. B. An advanced stage, showing the characteristic chain of secondary yellow spots, with rot well advanced at the base. Photograph of colored plate by Armena Eller.

Based on the assumption, from analogy, that the vector of yellow spot might be one of the homopterous bugs, suspicion at this time centered upon the three-cornered alfalfa hopper, *Strictocephala festina* (Say). This insect was first reported in the Islands in 1925, so this fact appeared to strengthen the hypothesis that it was the vector and that our pineapple virus possibly came with it. Although this insect had rarely been observed in pineapple fields, it is a strong flier and could easily migrate long distances. The fact that it commonly lived and bred upon Crotalaria and other leguminous plants was also taken into consideration, for many such weeds occur in the sections where pineapples are grown. These hoppers were collected in vast numbers in alfalfa fields and were caged by hundreds with pineapple plants, together with Crotalaria suffering from a mosaic disease. The insects appeared to feed freely upon pineapple leaves, but they failed to survive in the cages. No infections resulted from the feeding punctures, so they, too, had to be rejected.

Microscopic study of the diseased pineapple leaf by Dr. Royal N. Chapman disclosed very tiny, characteristic punctures, usually present in or near the initial spot. The supposition for a time was that these punctures were made by the mouth parts of some sucking insect. Sections cut through them by Dr. M. B. Linford indicated that they were, however, the nidus of an egg of some very tiny insect. Thus the search for the vector became quite sharply defined. The insect evidently was considerably smaller than any of the homopterous bugs known to be present in the Islands. Hence, such bugs were now eliminated.

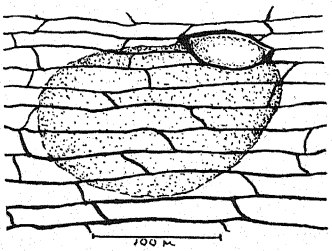


FIG. 9. Sketch of a thrips egg in pineapple leaf, magnified about 175 diameters. The puncture in the epidermis could be seen easily, but to observe the shadowy outline of the egg embedded in the mesophyll required a strong transmitted light.

We used tanglefoot extensively in the infected fields to trap transient insects, but even this method shed little new light on the subject. The same kinds of bugs were captured that had been collected on weeds. There was one addition, a minute Mirid, *Leucopoecila albofasciata* Reuter, which, for a time, became a suspect, chiefly due to the fact that members of the family Miridae are known normally to insert their eggs singly into plant

tissue. Then, again, this insect was so small that there was a possibility that its egg might fit the minute punctures. These bugs fed freely upon pineapple leaves, where they also oviposited. Measurements of the ovipositor, however, forced us to abandon this insect as a suspect, for its puncture was considerably too large. The region on the leaf where the egg was inserted developed a slight water-soaked appearance, with some discoloration, but there was no development of yellow spot.

About this time there first appeared evidence of reproduction of the disease in our cage experiments. Crotalaria plants showing marked mosaic yellowing and infested by aphids were collected in a field where yellow spot was present on pineapples. (I discovered later that the flowers were infested also with thrips.) These plants were potted, together with seedling pineapples, and enclosed in large lantern chimneys, October 7, 1929. November 4, a pair of initial spots began to show about 3 inches from the base on one of the pineapple leaves. As growth continued, the plant passed through all stages of the disease and finally rotted.

Hence it will be seen that the disease required almost a month to incubate before it made its appearance as a yellowing spot on the leaf. A second experimental plant was attacked with the disease after a period of 29 days with similarly infested Crotalaria. Plant lice are well-known vectors in some types of mosaic, but they could not possibly produce the punctures found so regularly in the vicinity of the initial spot.

We next turned our attention to a very tiny Anthrocorid bug, *Triphleps persequens* F. B. White, which preys upon aphids, thrips, and other insects. Great numbers were collected and caged on pineapple plants. They inserted their beaks and fed freely on the white portion of the leaf and also used their sword-like ovipositor to embed an egg here and there in the mesophyll. While these came much nearer to the size required than anything that we had had so far, they were still too large. Furthermore, none of the plants developed any sign of yellow spot.

Seeking a still smaller insect, I finally began an examination of the various species of thrips that occur in and about pineapple fields. One, referred to above in a preceding paragraph, in the flowers of diseased Crotalaria, is omnipresent on many field weeds. I suspected it as being the vector in the two instances cited. This species has a saw-like ovipositor (Fig. 10), with which eggs are inserted singly into the mesophyll of plants. With strong transmitted light the eggs embedded in the stamen tube of Crotalaria flowers were examined, and it was found that the punctures agreed perfectly both in size and shape with those that occur in diseased pineapple leaves. Hence, there appeared to be little question that we were at last on the right lead. Moreover, there was considerable evidence of

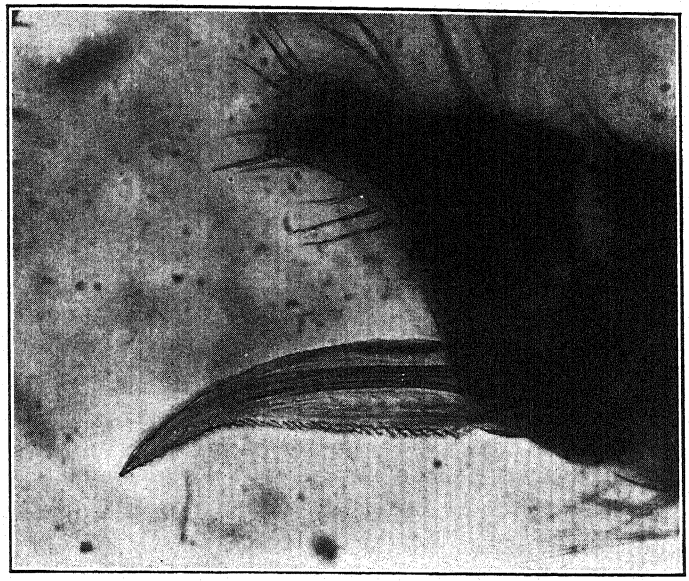


FIG. 10. The end of the abdomen of an onion thrips, *Thrips tabaci* Lind., showing the efficient, serrate ovipositor with which the eggs are inserted into the tissues of the plants. Highly magnified. Photograph by Dr. C. P. Sideris.

thrips feeding-punctures on the leaves of all affected pineapple plants. Infection, undoubtedly, is caused by the mouth parts. Ovipositing in the vicinity of the yellow spot is probably only incidental.

As a preliminary step, we collected thousands of thrips from various weeds and flowers in the affected localities and enclosed them over small seedling pineapple plants. Several species of thrips were concerned, but the onion thrips was remarkably scarce at the time, due to heavy rains. None was observed among those collected. Many of the thrips congregated between the imbricated leaves and evidently fed, for characteristic scars were present in the tender white epidermis. In several instances I located fresh eggs inserted into this same portion of the leaf. One of these eggs was sketched, using the camera lucida with a very strong, transmitted light projected through the leaf from below (Fig. 9). None of these plants developed the disease. After resigning from the Station, I continued the experiment, collecting thrips under more favorable weather conditions. At that time the onion thrips was much in evidence. Two of the pineapple plants used in the experiment soon developed the characteristic yellow spot. This evidence was very encouraging, pointing to thrips as the vector.

Though several species of thrips were included in this experiment, *Thrips tabaci*,[5] which occurs in the affected area on the leaves and in the

[5] This determination verified for me by Dudley Moulton in his letter of Feb. 18, 1930,

flowers of several weeds, became a strong suspect.[6] The recorded host plants of this species (2) include an extensive list of weeds and grasses. It bears, near the end of its abdomen, a strongly serrate ovipositor (Fig. 10), well suited to produce the punctures that we have noted.

As for records of thrips as a vector in the literature, I find nothing conclusive. However, there are several instances where these insects were thought to be responsible for the transfer of mosaic. Boning (1) had mosaic transmission from beets to spinach when only the onion thrips was in evidence. Another instance: Ogilvie (4) reported mosaic transmission on lilies, the only insects present being thrips (onion?) and a mealy bug. The same author (5) states that onion thrips can be controlled by two applications of nicotine sulphate, ½ pint to 50 gallons with 2 pounds of soap added. No results were obtained with nicotine dust. Triphleps, predaceous on these pests, checked them in May.

Several excellent papers dealing with the biology of onion thrips are available. The best of these are in a series by Wardle (6), MacGill (3), and Wardle and Simpson (7). These studies were made on cotton in the United States and Egypt. The observations of the authors that thrips injury was particularly destructive along windward edges with relatively little destruction to leeward fits exactly the situation with yellow spot pineapples. Thrips, being exceedingly small, are commonly distributed by the wind. Their wings are so feather-like that it is impossible for them to make progress even into a light breeze. Again, it was found in the cotton investigation that heavy rains materially cut down thrips injury, since these pests are so poorly protected. This observation, too, just fitted the situation we had during January, 1930. No yellow spot appeared in our fields following the heavy rains. Furthermore, it was observed on cotton that drought and high temperatures of summer slowed down the thrips injury to the lowest ebb. This, too, fits for yellow spot of pineapples. In Egypt, thrips were found to be most prolific during periods of light showers and moderate weather. It is noted that yellow spot has been most destructive under such conditions (Fig. 4, Graphs for 1926, 1928, and 1929).

COMBATING THRIPS ON PINEAPPLES

Control measures, of course, are only tentative at this time. Since yellow spot on pineapples probably comes from weeds affected with mosaic, clean culture is of prime importance. With this crop thrips do their dam-

[6] I have since learned that Dr. M. B. Linford, who took over the investigation, concurrently reproduced the disease and subsequently has established final proof of its relationship to the disease, as reported in the Station's files and in a paper accepted for publication in *Science* as a preliminary report.

age down in the axils of the leaves. To reach them it is necessary to treat only the heart of the plant. Theoretically, the insects could be kept out with some sort of a mechanical plug. Various substances have been tried, including tobacco dust, wheat bran, rice hull, volcanic black sand, etc. A deterrent added to these, composed of an oil emulsion with tobacco extract, increases efficiency. Dusting sulphur, used with success against thrips in California, has good possibilities. The combination of tobacco and sulphur, under the trade name Nico-sulfur, is also being tried. Tobacco extract, used alone, evidently volatilizes too quickly, for it has not shown results.

Weather conditions influence greatly the need for control measures. As indicated in earlier paragraphs, both heavy rains and droughts appear to be fatal to thrips. Natural enemies, too, have an important bearing upon their control. In the United States, where these have been studied, we learn that several species of ladybird beetles and their larvae prey upon thrips. The various species of Triphleps also are well-known predators of these pests. Many other insect enemies, including parasites, have been recorded, but information dealing with them is meager.

In Hawaii we find *Triphleps persequens* omnipresent in flowers or anywhere that thrips congregate. As soon as these bugs become abundant in spring, thrips become scarce. Another important predator, everywhere in pineapple plants, is the Cucujid beetle, *Cryptamorpha dejardinsi* Guer. Both the adults and their larvae live (deep down) in the axils of the leaves, where they prey upon any small insects that come in their way. Without such friends the growing of crops would be quite impossible.

SUMMARY

A mysterious new disease of pineapples appeared in Hawaii during 1926. It usually was evidenced by a breaking-down of the tissues at the base of one or more leaves, the stem soon bending over in the direction of the injured part; the whole plant eventually succumbing. This led to the name side rot. From the subsequent discovery that an initial yellow spot on one or more leaves usually preceded the rotting at the base, the name was changed to yellow-spot disease.

This disease was most troublesome on newly planted tops because of their open structure. Soon it was found to attack these even while attached to the fruits. All attempts to control the disease with sprays proved futile.

Each year the disease increased, and it spread widely in the Islands. During the fairly dry season of 1929 it was more than tenfold what it had been in previous years. At that time it was assumed that it might be a virus disease. It was soon learned that it could not be reproduced by artificial means. Then began a search for the vector among the numerous in-

sects associated with the plants. Finally, with large numbers of the common insects eliminated, the search was narrowed down to one whose characteristics, even before its discovery, were sharply defined by histological studies on the part of other workers on the problem. At the time this paper was written a species of thrips was a strong suspect. I am informed that the final proof of its relationship to the disease has been established by Dr. M. B. Linford and has been accepted for publication as a preliminary report in *Science*.

FORMERLY WITH THE EXPERIMENT STATION,
ASSOCIATION OF HAWAIIAN PINEAPPLE CANNERS,
HONOLULU, HAWAII.

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BACTERIA ANTIBIOTIC TO USTILAGO ZEAE[1]

R. H. BAMBERG[2]

INTRODUCTION

In the spring of 1928 the writer began an investigation of the factors affecting the infection of corn plants with *Ustilago zeae* (Beck.) Ung. During the course of the field work in the summer of 1928 it became evident that young plants seldom became infected and that many older plants which had been artificially inoculated failed to become infected. Discolored areas often developed near the point of inoculation, when the inoculations were made with hypodermic needles. Cultures were made from these discolored areas, and bacteria were isolated that proved to be decidedly antibiotic to *U. zeae*. Because of the fact that this bacterial culture often seemed to prevent infection, even when susceptible corn plants were inoculated by standard methods with virulent cultures of *U. zeae*, this phase of the problem was further investigated. The results are presented in the following pages.

SOURCES OF CULTURES

Several cultures of bacteria were isolated in the summer of 1928 from corn plants that had been inoculated with *Ustilago zeae* but had failed to develop smut galls. Instead of smut galls, which usually develop on plants inoculated with virulent lines of smut, there were brownish rotted areas on the plants around the inoculation courts. The cultures were obtained from the inner tissues of these rotten areas. Transfers were made with a sterile needle from these diseased tissues to Petri dishes containing 1.5 per cent potato-dextrose-agar. Transfers then were made from resulting colonies to potato-dextrose-agar slants, and these were used as stock cultures. All cultures were grown on potato-dextrose-agar and kept in the laboratory at room temperature unless otherwise stated. Cultures B–1, B–2, B–3, B–4, B–5, and B–6 were obtained in this way. Other cultures of bacteria were obtained from contaminations that appeared in smut colonies that developed from chlamydospores taken directly from smut galls. Still others were procured from contaminations that appeared in stock cultures in the laboratory. Bacterial cultures B–7, B–8, B–9, B–10, B–11, B–12, B–13, B–14 and B–15 were obtained in this way.

[1] Paper No. 963 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writer wishes to express his appreciation for the assistance given by Dr. E. C. Stakman, under whose direction these investigations were made. He also wishes to express his indebtedness to Dr. J. J. Christensen for many helpful suggestions and criticisms.

A combination of 4 monosporidial lines of *Ustilago zeae* was used for all inoculations made on corn. Three of these lines were of one sex and one of another, and the combination of these 4 lines had produced heavy infection in previous pathogenicity tests.

Sporidia were obtained by growing the 4 lines of smut on a potato decoction containing 1 per cent malt and 1 per cent dextrose. An abundance of sporidia was produced on this medium. The smut cultures were grown separately until they were to be used. The bacteria were grown in a similar medium. They were mixed with the smut cultures by adding from 5 to 10 cc. of the suspension of bacteria to approximately 50 cc. of the mixture of lines of smut.

Inoculations on corn plants 8 to 10 inches high were made in the greenhouse by hypodermically injecting about ½ to 1 cc. of the suspension of sporidia or of a mixture of sporidia and bacteria into each plant at each of 3 places, namely: (1) at the surface of the ground, (2) about 1 inch above the ground line, and (3) about 1½ to 2½ inches above the ground line. Most of this inoculum remained between the tightly rolled leaves, but some of it was lost by being forced up between the leaves to the outside.

EFFECT OF ANTIBIOTIC BACTERIA ON CULTURES OF USTILAGO ZEAE AND OTHER SMUTS

Colonies of *Ustilago zeae* in test-tubes and in Erlenmeyer flasks were inoculated, by transferring a small amount of bacteria with a sterile needle to a point on the margin of the colony, with bacterial cultures B–1, B–2, B–3, B–4, B–5, and B–6. Colonies about 1 inch in diameter were completely surrounded by the bacterial growth in from 3 to 5 days, and the further growth of the fungus was stopped. Before inoculation the surface of smut colonies was rather dry but became wet and slimy as the bacteria grew and spread. The action on colonies of smut appeared to be very similar for all 6 of these bacterial cultures. For more intensive study only 1 culture, B–1, from these isolations was used.[3]

Colonies of *Ustilago zeae* that had been growing on potato-dextrose-agar in 250 cc. flasks for 9 days were inoculated with culture B–1. In 6 or 7 days the cultures of smut were changed from compact colonies that could all be lifted from the medium with an inoculating needle to slimy colonies of which only a small portion could be lifted with a needle. The colonies of smut were changed to slimy masses with very little or no fungus growth left. The appearance of the colonies is shown in figure 1. These experiments were repeated many times and the results were in general always the same.

[3] The purity of this culture was not established, but it was turned over to Dr. Delia Johnson for further work.

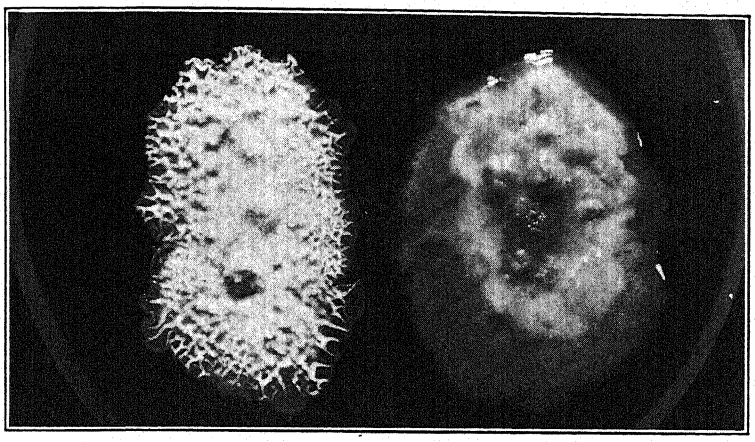


FIG. 1. Seventeen-day-old cultures of *Ustilago zeae* growing on potato-dextrose agar. The colony on the right, when 9 days old, was inoculated with bacterial culture B–1.

Colonies of smut in test-tubes appeared to be affected the same as those in flasks. Tough cultures of the smut fungus seemed to be almost completely dissolved. When examined microscopically only a very few sporidia and segments of mycelium were left in the colonies of *Ustilago zeae* that had been inoculated with B–1, while in pure cultures of smut of the same age there was almost a solid mass of sporidia and mycelium.

Colonies of *Ustilago zeae,* approximately 1 to 1½ inches long, on agar slants were inoculated on the lower margin with B–1 and 4 colonies incubated at each of the temperatures 10, 15, 20, 25, and 30° C. The bacteria were able to destroy the smut colonies at all these temperatures but the lytic effect was most rapid at 25° and 30° C. Figure 2 shows the average rate of advance of the bacteria through the fungus colonies.

Colonies of *Ustilago zeae* on agar slants were also inoculated with bacterial cultures B–7, B–8, B–9, B–10, B–11, B–12, B–13, B–14, and B–15 to determine the effect of these other bacteria on the growth of smut colonies. Four of these bacteria greatly retarded or prevented further development of the colonies of smut, but none caused such complete dissolution as did B–1 and the other cultures from the same source. Colonies of *U. zeae* inoculated with the other 5 bacteria continued to grow normally and increase in size, although culture B–11 seemed temporarily to delay the development of a young culture of smut, which later developed normally.

These results indicate that some bacteria completely prevent growth of *Ustilago zeae* on solid culture media and may even destroy fully developed colonies, while other bacteria have no appreciable effect.

Cultures of *Ustilago zeae* 6 days old, growing in malt-dextrose-potato decoction, were inoculated with B–1. At the time of inoculation, mounts

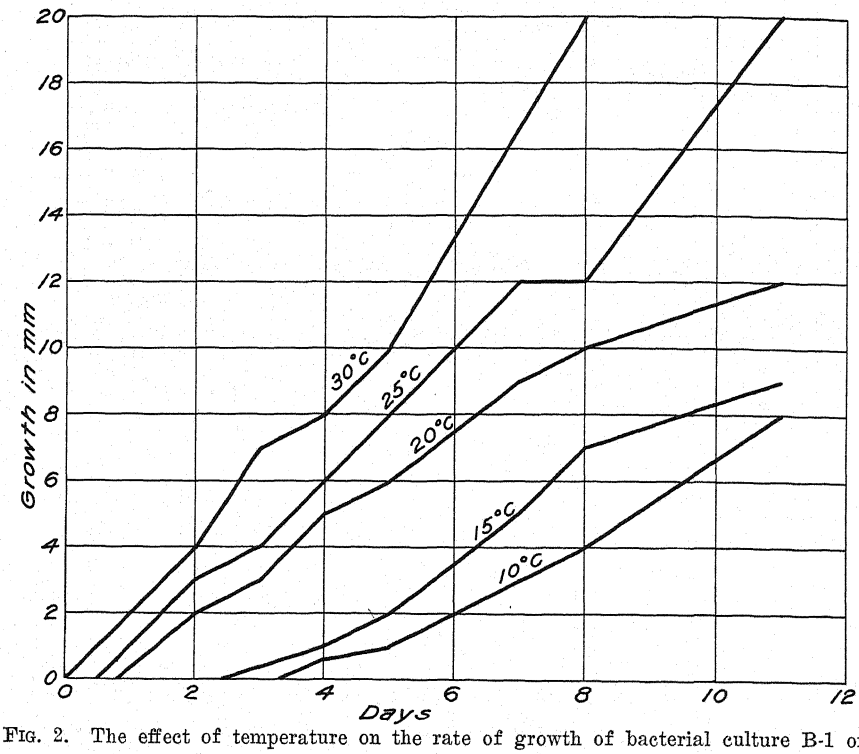


FIG. 2. The effect of temperature on the rate of growth of bacterial culture B-1 on colonies of *Ustilago zeae*, as measured by the length of the bacterial colonies at different intervals of time.

of the sporidial suspension were examined under the microscope. A large number of sporidia were found in all the flasks. Ten days later the flasks inoculated with B–1 contained a much smaller number of sporidia of *U. zeae* than did the flasks which contained the pure cultures of *U. zeae*. These experiments were repeated a number of times, with the same general results. There might have been two reasons for the smaller number of sporidia in the flasks inoculated with the bacteria: (1) direct action of the bacteria on the sporidia, resulting in their disintegration, and (2) production of an environment unfavorable to the development of the smut fungus.

In order to determine whether the bacteria were directly responsible for the disintegration of the sporidia a large number of hanging drops of a sporidial suspension of *Ustilago zeae* were set up in van Tieghem cells and inoculated with B–1. Other drops in cells on the same slides were set up and left uninoculated as checks. The sporidia in these hanging drops were observed through the microscope at frequent intervals. After about 24 hours the number of sporidia in the drops not inoculated with the bacteria

was considerably greater than in the inoculated drops. After about 36 hours a mass of new sporidial growth was clearly visible with the naked eye on the surface of drops not inoculated with B–1, while no such growth could be seen on any of the drops inoculated with B–1. Instead of an increase in the number of sporidia in the hanging drops inoculated with B–1 there was a rather rapid decrease in most cases. With this decrease in number of sporidia what appeared to be particles of disintegrated sporidia could be seen in some of the drops that had been inoculated with B–1. These particles were irregular bodies of no definite shape, usually larger than the bacteria but smaller than the sporidia. Similar particles could not be seen in any of the drops not inoculated with B–1.

Since the bacteria had a marked antibiotic effect on cultures of corn smut, an attempt was made to determine the effect of one of these cultures on some other smuts. Five colonies of *Ustilago avenae* and 2 of *U. levis* in test-tubes were inoculated with B–1. Seven days after inoculation all growth apparently had been stopped. In 4 weeks the tough mycelial colonies were changed to a rather slimy mass and the dissolution seemed almost as complete as in the case of *U. zeae*.

The inoculations of *Ustilago avenae* and *U. levis* were repeated, using 3-day-old colonies of both smuts. Four cultures each of 2 forms of *U. avenae* and 1 form of *U. levis* were inoculated with B–1, and 2 cultures of each were used as controls. The colonies used as controls continued to develop rapidly, but those inoculated with B–1 were reduced to a slimy mass in 5 days.

Inoculations of B–1 on *Sorosporium reilianum* (Kühn) McAlp. and *Tilletia tritici* (Bjerk.) Wint. indicate that the bacteria are destructive to colonies of *S. reilianum* and very injurious to the growth of *T. tritici*. The colonies of *S. reilianum* seem to be dissolved fairly readily. Colonies of *T. tritici* were prevented from further growth, but dissolution of the colony took place very slowly. This may have been because the colonies were very old and tough before they were inoculated with B–1.

The effect of the bacteria on infection of corn with Ustilago zeae. Laboratory investigations indicated that the growth of *Ustilago zeae* in pure culture was greatly retarded or prevented by certain cultures of bacteria and that the sporidia were disintegrated by them. Therefore, experiments were made in the greenhouse to determine the possible effect of the bacteria on the infection of corn plants by *U. zeae*. Sporidial suspensions of 4 monosporidial lines of *U. zeae* were grown separately in flasks containing malt-dextrose-potato decoction. These monosporidial lines were mixed together by straining into a large flask through cheesecloth to remove any material that would not pass through a hypodermic needle. This mixture

of smut lines was then divided into 2 equal parts and a suspension of B–1 in the same kind of medium added to one half at the rate of 1 part of bacterial suspension to 2 parts of smut inoculum. Another type of inoculum used was a mixture containing the same 4 monosporidial lines of smut and bacterial culture B–11. Treatments with distilled water were made as controls.

On November 3, 1928, a number of plants of Golden Bantam corn, 8 to 10 inches high, growing in beds of soil in the greenhouse, were inoculated with each of the different kinds of inoculum given in table 1. Notes, as summarized in table 1, were taken on November 23.

TABLE 1.—*The effect of bacterial cultures B–1 and B–11 on the development of smut in corn plants inoculated with Ustilago zeae*

Material injected	Number of plants		Percentage of infection
	Inoculated	Infected	
Distilled water	95	0	0
Four monosporidial lines of *U. zeae*	104	75	72.1
Four monosporidial lines of *U. zeae* plus B–11[a]	103	74	71.8
Four monosporidial lines of *U. zeae* plus B–1	102	8	7.8

[a] In this case bacterial culture No. B–11 was grown in association with 1 monosporidial line of the corn smut.

Two plants inoculated with *Ustilago zeae* and B–1 were decayed in the bud, very much like those from which the bacteria were first isolated. Several isolations of bacteria were made from these 2 plants. These appeared to be the same as the original cultures used as inoculum. Colonies of *U. zeae* in test-tubes inoculated with these reisolated bacteria were destroyed.

Further inoculations were made into Gehu Flint corn on February 18, 1929, using only the 4 monosporidial lines of *Ustilago zeae* and the 4 lines plus B–1. Notes were taken March 3. The results, given in table 2, are similar to those for the previous inoculations.

TABLE 2.—*The effect of bacterial culture B–1 on the development of smut in corn plants inoculated with Ustilago zeae*

Inoculum	Number of plants		Percentage of infection
	Inoculated	Infected	
Four monosporidial lines of *U. zeae*	55	29	52.7
Four monosporidial lines of *U. zeae* plus B–1	105	8	7.6

Tables 1 and 2 show rather conclusively that, when B–1 is mixed with the smut at the time of inoculation, the percentage of infection by *Ustilago zeae* is greatly reduced. Table 1 further shows that certain other bacteria, such as culture B–11, did not reduce the amount of infection.

In order to determine the effect of the bacteria on the development of the smut when injected into the plant at times other than that of inoculation with *Ustilago zeae,* a series of inoculations with bacteria was made before and after the time of injection with the suspension of sporidia. The first inoculations with bacteria were made 5 days before those with the smut. When B–1 and *U. zeae* were injected the same day the suspension of bacteria and sporidia were mixed together before injection. Readings on infection percentages were made April 7, 1929. The results of the inoculations are given in table 3.

There were 3 rather distinct types of infection and the percentage of each caused by the different kinds of inoculum is given separately. In some plants there was either chlorosis or red coloration of the leaves without the formation of definite galls or warty thickenings on the leaves. Plants having this type of infection were classed under "chlorosis or anthocyanin coloring." In some there were wart-like thickenings on the leaves or sheaths. Rounded galls with spores had not fully formed. These were classed as "incipient galls." Under "large galls" were placed those plants that had well-formed galls containing spores.

These results indicate that the effect of B–1 on the development of *Ustilago zeae* in the host is more pronounced when injected into the plant at the same time as the smut but that a considerable influence of the bacteria is retained when they are injected for at least 3 days prior to inoculation with smut. Apparently, the amount of infection is significantly reduced when the plants are injected with B–1 1 and 3 days after inoculation with smut. This is especially true if one considers the severity of infection as indicated by the size of galls and extent of chlorosis.

Even after smut galls have attained considerable size they may be disintegrated by the action of the bacteria and prevented from forming spores. Thirty or more galls $\frac{1}{4}$ inch to $\frac{3}{4}$ inch in diameter were inoculated on the plants by injecting a small amount of bacterial suspension into them with a hypodermic needle. Almost all the galls dried up after 2 weeks, without producing chlamydospores. Forty other galls, about the same size as those inoculated with B–1 on the plants, were placed in Petri dishes with portions of the host plants attached. Two large galls were put in each Petri dish. One was inoculated with B–1 by pricking with a needle which had been dipped into a colony of the bacteria; the other was pricked with a sterile needle. In 24 hours a slimy exudate came from 2 of the inoculated

TABLE 3.—*The effect of inoculating corn plants with Ustilago zeae at different periods after the plants had been inoculated with bacterial culture B–1 and at different periods preceding the bacterial inoculations*

Inoculations		Plants inoculated	Total infections	Plants with		
Inoculum	Date			Chlorosis or anthocyanin coloring	Incipient galls	Large galls
		No.	Per cent	Per cent	Per cent	Per cent
B–1 *U. zeae*	March 18, 1929 March 23, 1929	101	79.2	22.8	28.7	27.8
B–1 with *U. zeae*	March 23, 1929	106	56.6	35.8	12.2	8.4
B–1 with *U. zeae*	March 23, 1929	102	48.0	40.2	6.8	.9
U. zeae	March 23, 1929	118	90.6	33.9	27.1	29.6
U. zeae	March 23, 1929	103	81.5	20.4	21.3	39.8
U. zeae B–1	March 23, 1929 March 24, 1929	122	73.7	37.7	18.8	17.2
U. zeae B–1	March 23, 1929 March 26, 1929	116	68.1	31.9	22.4	13.8
U. zeae B–1	March 23, 1929 March 28, 1929	113	77.0	30.9	26.5	19.4
U. zeae B–1	March 23, 1929 March 30, 1929	106	83.9	23.5	26.4	34.0

galls. In 4 days all of the galls inoculated with B–1 were almost completely disintegrated.

These investigations with *Ustilago zeae* in culture and in the host plant have shown rather conclusively that certain cultures of bacteria, and 1 culture in particular, designated B–1 for convenience, in some way have a deleterious effect on the development of the smut both in cultures and in the host. An attempt was made to determine whether it was the direct effect of the bacteria on the smut fungus or the effect of some by-product of the metabolism of the bacteria. Two cultures of the bacterium, B–1, grown on malt-dextrose-potato decoction, 1 for 4 and the other for 7 days, were filtered through sterile Berkefeld filters (medium). Colonies of *U. zeae* in test tubes to which a loop of the filtrate was transferred continued to grow apparently the same as control colonies to which no filtrate had been added. No growth appeared on agar slants to which loops of the filtrate were transferred. Part of the filtrate was poured into a mixture of

sporidia of 4 lines of *U. zeae* at approximately the ratio of 1 part of the filtrate to 4 parts of the sporidial suspension, and the whole allowed to stand 24 hours. Table 4 gives the results of the inoculation of plants with this mixture as compared with inoculation with the same lines mixed with B–1. Apparently, there was nothing in the filtrate which inhibited or retarded the development of *U. zeae*.

Antibiosis between microorganisms has been observed by many workers. A general review of the literature dealing with the phenomenon, given by Buchanan and Fulmer,[4] indicates that the action is quite variable. This aspect of the problem will be considered in more detail in a paper by Delia Johnson.

TABLE 4.—*The ineffectiveness of a sterile filtrate from B–1 on the development of U. zeae*

Inoculum	Plants inoculated	Infections
	No.	Per cent
U. zeae + filtrate from B–1	37	72.9
U. zeae + B–1	33	0

DISCUSSION AND CONCLUSIONS

Certain cultures of bacteria were found to inhibit the development of *Ustilago zeae*. Experiments proved that these bacteria were capable of preventing the multiplication of sporidia of *U. zeae* and of destroying colonies of *U. zeae* and of some other smuts already formed in culture. The wide-spread distribution of such bacteria may serve as an important check on the multiplication of inoculum in the soil.

A culture of bacteria which prevented the growth of *Ustilago zeae* in culture also greatly reduced the percentage of smut infection when injected into the host along with the sporidial suspension. In one of the pathogenicity tests the percentage of infected plants was 72.1, where a combination of 4 monosporidial lines of smut was used as inoculum, in contrast to 7.8 where the same combination of smut lines mixed with the bacteria was used. The fact that certain bacteria do prevent smut infection of corn plants to a great extent may account for many of the irregularities observed in the percentage of infection resulting from artificial inoculations. It may also account for many of the variations in the amount of smut oc-

[4] Buchanan, R. E., and Ellis I. Fulmer. Physiology and biochemistry of bacteria. Vol. III, 575 pp. Williams and Wilkins Co., Baltimore. 1930.

curring under natural conditions in different fields in the same year and in the same field in different years.

The destructive action of such bacteria as were found to destroy smut galls already formed on corn plants may account for the disappearance of smut on corn plants in the field. The presence of such bacteria also may account for the appearance of abortive galls often observed. Corn smut, attacked early in its development on the host, may fail to develop normally.

SUMMARY

1. Ten cultures of bacteria were found to have a deleterious effect upon the development of *Ustilago zeae* in culture, while 5 others had no observable like effect.

2. Sporidia of *U. zeae* apparently failed to multiply in the presence of a culture of bacteria isolated from corn plants which failed to produce galls when inoculated with *U. zeae*.

3. Evidence of disintegration of sporidia by the bacteria was obtained.

4. A combination of monosporidial lines of *U. zeae* was made less virulent in its attack on the host by the association with these bacteria.

5. The injurious effect of the bacteria on the development of the smut in the host persisted when injected into the corn plant at least 3 days before inoculation with *U. zeae*. Apparently, the bacteria were able to live in or on the corn plant at least that long.

6. The virulence of the smut seemed to be significantly reduced even when the bacteria were injected into the plants as much as 3 days after inoculations with smut.

7. Smut galls were disintegrated and spore formation was prevented even after distinct galls more than $\frac{1}{2}$ inch in diameter had been formed.

8. The destructive action seems to be directly associated with the presence of the bacteria, as the filtrate from cultures of the bacteria had no appreciable effect on colonies of smut in culture or on the development of smut galls in inoculated plants.

FIELD STUDIES ON THE RING-SPOT DISEASE OF BURLEY TOBACCO IN WASHINGTON COUNTY, VIRGINIA[1]

S. B. FENNE[2]

INTRODUCTION

Tobacco ring spot is a virus disease that is becoming more and more prevalent in the State every year. In 1917, when the disease was first observed by Fromme and Wingard, it was considered of minor importance. Infection at that time was observed on only an occasional plant in a field and the crop loss was negligible. The amount of infection, however, has increased from year to year until now it is not uncommon to find fields in which 90 per cent of the plants are infected.

The writer studied this disease under field conditions in 10 counties of the State during the summer of 1927 and made a special study of it on Burley tobacco in Washington County during the seasons of 1928, 1929, and 1930. The disease was first described and illustrated as ring spot by Fromme and Wingard.[3]

Fromme, Wingard, and Priode[4] recorded ring spot in 11 counties in Virginia. They proved that the ring-spot disease was infectious and concluded that it should be classed with the virus diseases. Wingard[5] reported that the ring-spot virus had a very wide host range, although it was very specific in its infective properties, since many plants that were inoculated failed to develop the disease. He was the first to find natural ring-spot infection on any plant other than tobacco. Repeated inoculations on tobacco, with expressed juice from sweet clover, produced typical ring-spot symptoms.

MATERIALS AND METHODS

To determine if certain weeds were harboring the ring-spot virus, suspected plants growing in or around the plant beds and fields were sent to Dr. Wingard at the Virginia Agricultural Experiment Station for inoculation tests on healthy tobacco plants.

[1] Condensed from a thesis presented to the Department of Botany and Plant Pathology of the Virginia Polytechnic Institute in partial fulfillment of the requirement for the degree of Master of Science.

[2] The writer is particularly grateful to Dr. S. A. Wingard, under whose direction this work was done, for correcting the manuscript and for invaluable suggestions, advice, and encouragement.

[3] Fromme, F. D., and S. A. Wingard. Blackfire or angular leaf-spot of tobacco. Va. Agr. Exp. Sta. Tech. Bul. 25. 1922.

[4] Fromme, F. D., S. A. Wingard, and C. N. Priode. Ring-spot of tobacco, an infectious disease of unknown cause. Phytopath. 17: 321–328. 1927.

[5] Wingard, S. A. Hosts and symptoms of ring-spot, a virus disease of plants. Jour. Agr. Res. 37: 127–153. 1928.

The insects studied were secured from the writer's vegetable garden and placed on ring-spot-infected tobacco plants in cages and left for different periods of time. They were then transferred to caged healthy tobacco plants. Tobacco flea beetles, *Epitrix parvula* Fabr., were secured from tobacco plants. Potato or cucumber flea beetles, *Epitrix cucumeris* Harr., and leaf hoppers, *Empoasca fabae* Harr., were secured from potatoes. Aphis, *Macrosiphum solanifolii* Ashm., were secured from potatoes and clover. The tobacco worm, *Phlegethontius quinquemaculata* Haworth, and the firefly, *Photinus scintillans* Say, were found on tobacco plants. The insect catcher described by Kunkel[6] was used.

The extent of injury to affected plants was determined by counting and measuring the leaves on diseased and healthy plants at topping time. The quality of the leaf also was taken into consideration. Numerous inspections and field counts of affected plants gave the number of plants diseased. The total loss to the county was estimated by multiplying the average extent of injury to the individual plant by the total percentage of affected plants. Judy's Pride, Kelley's, and Lockwood varieties of tobacco were used in all experiments conducted in Washington County.

EXPERIMENTAL RESULTS

Since ring spot is becoming of such great economic importance and so little is known regarding it, experiments were undertaken in an effort to determine the source of inoculum, means of dissemination of inoculum, the rate of spread of disease in the field, the extent of injury to affected plants, the percentage of plants affected, and the loss to the county.

SOURCE OF INOCULUM

Four possible sources of inoculum suggested themselves: (1) seed, (2) insect vectors, (3) weed hosts, and (4) soil.

Seed: In studying the possibility of the transmission of ring-spot virus by tobacco seed, plant beds in 6 counties in Virginia were inspected in 1927. In only 3 plant beds in 1 county was ring spot found, and this was late in the season, after all transplanting had been completed. Therefore, it is safe to assume that the infection did not necessarily originate within the plant bed.

In 1928, 1929, and 1930, close observation was kept on seed beds in Washington County. They were visited at short intervals until transplanting time. In 1928 and 1929, no ring-spot infection was found in the plant beds. In 1930, however, plant-bed infection was observed in 10 per cent of the beds inspected in Washington County. This observation was surprising, since plant-bed infection had been regarded as very rare up to that time. During the same season, however, plant-bed infection was re-

[6] Kunkel, L. O. Studies on aster yellows. Amer. Jour. Bot. 13: 646–705. 1926.

ported by Godkin[7] in one other county in Virginia and by Valleau[8] in Kentucky.

This opens up a new problem formerly thought settled. Did the plant-bed infection arise from infected seed, or were the young plants inoculated by some insect that carried the inoculum from near-by weed hosts, which were in all cases very abundant?

Insect studies: Because of the similarity between ring spot and certain insect-borne virus diseases, it was thought advisable to study certain insects as possible vectors of the ring-spot virus. The tobacco flea beetle attacks tobacco plants both in the plant bed and in the field. For this reason it was looked upon as a possible disseminator of the ring-spot virus. On June 11, 75 flea beetles were collected from healthy tobacco plants and placed in cages on ring-spot-affected tobacco plants. On June 22, 15 of these beetles were transferred to 2 healthy tobacco plants in an insect-proof cage. On June 24, 30 more of these beetles were removed and placed on healthy tobacco plants in insect-proof cages. In this case 15 beetles were placed on each of 2 plants in separate cages. On July 6, the beetles were alive and thrifty, but no ring-spot infection had developed. On August 2, the plants were normal and free from ring spot.

On July 4, 15 flea beetles were transferred from ring-spot-infected tobacco plants in the field to a healthy tobacco plant in an insect-proof cage. This plant was examined on August 24 and found to be normal in appearance. On July 5, 15 flea beetles that had fed on diseased tobacco plants for 25 days were transferred to a caged healthy tobacco plant. On August 24, no ring spot had developed. These experiments seem to show that the ring-spot virus is not transmitted by tobacco flea beetles.

On June 11, 75 potato flea beetles were collected from potato plants and placed in an insect-proof cage on ring-spot-infected tobacco plants. On examination, 8 days later, all of these beetles were dead.

On June 19, 50 adult and nymph aphids, *Macrosiphum solanifolii,* were placed on diseased tobacco plants in an insect-proof cage. Seven days later all aphids were dead. Apparently, tobacco is not a favorable host for these aphids.

On June 11, 5 tobacco worms were placed in a cage on an infected plant. On July 4, these worms were transferred to a healthy caged plant. On August 24, no ring spot had developed.

On June 24, 50 leaf hoppers were placed on diseased tobacco plants. On July 4, 10 days later, all leaf hoppers were dead. On July 4, 15 leaf

[7] Godkin, James. Tobacco plant bed survey in Virginia. U. S. Dept. Agr., Bur. Pl. Indus. Plant Disease Reporter 14: 121. 1930.

[8] Valleau, W. D. Tobacco seed beds in Kentucky. U.S. Dept. Agr., Bur. Pl. Indus. Plant Disease Reporter 14: 113. 1930.

hoppers were transferred from potatoes in the field to a healthy tobacco plant in a cage. On August 2, these leaf hoppers also were dead and no infection resulted.

It seems that some strong flying insect might be responsible for disseminating ring spot since infection was first observed in scattered parts of the fields with apparently no relation to earlier affected plants. The only strong flying insects observed on tobacco were the common fireflies, but in my cage experiments they died after a few days' confinement.

Weed hosts: Many perennial weeds have been found susceptible to the ring-spot disease and many of them, such as the horse nettle and pokeweed, are common in and around tobacco fields. In order to determine whether such plants harbored the virus, suspected specimens were sent to Dr. Wingard to be used as inoculum on healthy tobacco plants. Sweet clover, *Melilotus alba* Desr., on several occasions, produced typical ring-spot symptoms when inoculated on healthy tobacco plants. In July, 1929, the writer sent Dr. Wingard several suspected weeds in which were included stick weed or yellow crown beard, *Verbesina alternifolia* Britton. Healthy tobacco plants were inoculated with the expressed juice from this plant. Nine days later very distinct ring-spot infection was found on the inoculated plants. The stick weed was slightly yellowish with the upper leaves distorted, and rosette-like. No unusual spotting on the leaves was noticeable. Stick weed has since been found by the writer to be a common carrier of the ring-spot virus.

Other plants sent to Dr. Wingard were: prickly lettuce, *Lactuca virosa* L.; broad leaf plantain, *Plantago major* L.; burdock, *Arctium lappa* L.; pokeweed, *Phytolacca decandra* L.; milkweed, *Asclepias* sp.; European bittersweet, *Solanum dulcamara* L.; smart weed, *Polygonum* sp.; lamb's-quarters, *Chenopodium album* L.; broad-leaf dock, *Rumex obtusifolius* L.; button weed, *Malva rotundifolia* L.; Jimson weed, *Datura stramonium* L.; red clover, *Trifolium pratense* L.; mammoth clover, *Trifolium medium* L.; alsike clover, *Trifolium hybridum* L.; and white clover, *Trifolium repens* L.

These plants were sent in at different periods of the year, and some species at several different times. Suspected plants, not only from Washington County but also from Halifax, Amherst, Nelson, Appomattox, Pittsylvania, Mecklenburg, Charlotte, Brunswick, Dinwiddie, and Montgomery counties were used. Negative results were obtained in every case in these tests.

Soil: An experiment was conducted on a large scale on one farm to determine whether the plant-bed soil could transmit the disease. Five hundred square yards of plant-bed soil were thoroughly steam sterilized. As

a result, the plant beds were very clean and free from weeds. The plants were very thrifty and vigorous. Nevertheless, 30 per cent of the plants in fields set from this plant bed developed ring-spot infection. This seems to prove conclusively that the ring-spot infection in this case did not originate in the plant-bed soil.

RATE OF SPREAD IN THE FIELD

After tobacco is set in the field it has been noticed that in some instances a very rapid spread of ring spot occurs. In order to study the rate of spread in the field, inoculations were made in 7 fields and observations made on the rate of spread of infection, at 10-day intervals, through the growing season.

In each case a single tobacco plant was inoculated with the virus, typical ring-spot lesions appeared in every case on the inoculated plants, and the infection became systemic, with all leaves showing symptoms of the disease.

In some cases ring spot was observed in various parts of the field the following day; but, in other cases, it was not observed until 20 days later. In some fields it was found in close proximity to the inoculated plant and in other cases the infection was first noted in remote parts of the field. Affected plants were observed in a very irregular order with apparently no relation to the plant originally inoculated. It, therefore, seems that the infection came from some other source, such as weed hosts. Various kinds of weeds were present in great abundance, surrounding every field in which a spread of ring-spot infection was noted.

PERCENTAGE OF PLANTS AFFECTED

One hundred and seventy-one fields in 10 counties and 49 plant beds in 6 counties were inspected in 1927. An average of 2.5 per cent ring-spot infection was found in the 171 fields observed. In 1 field in Brunswick County ring spot occurred on 75 per cent of the plants.

Fifty representative tobacco fields were examined in 1928 in Washington County. Ring spot was found in 46 of these. An average of 3 per cent infection was recorded for that year.

One hundred tobacco fields in different parts of the county were inspected in 1929. Only 2 small fields were found that did not contain ring spot. Many fields were found with from 50 to 90 per cent infection, but the majority contained from 2 to 12 per cent, with an average of 7.6 per cent. The highest percentage of infection was found in the middle of the growing season, when the plants were about a foot high. At topping time the ring-spot symptoms had been masked on about 50 per cent of the affected plants in most fields.

EXTENT OF INJURY TO THE AFFECTED PLANTS

It is very important to know the extent of injury to affected plants by ring spot; therefore, the leaves on 20 healthy and 20 diseased plants were measured at topping time. The number of leaves, the dimensions of the leaves, the extent of tissue injury, and the quality of the leaves on each plant were determined. The results of this study are shown in table 1.

TABLE 1.—*Average size of leaves on healthy and diseased plants*

Healthy plants		Diseased plants	
Plant No.	Average leaf dimensions in inches	Plant No.	Average leaf dimensions in inches
1	27 × 13	1	19 × 8
2	27 × 14	2	20 × 9
3	23 × 9	3	17 × 9
4	27 × 11	4	25 × 12
5	27 × 13	5	28 × 11
6	27 × 12	6	23 × 11
7	25 × 12	7	23 × 10
8	22 × 11	8	19 × 7
9	28 × 13	9	26 × 12
10	24 × 10	10	24 × 10
11	21 × 12	11	23 × 8
12	26 × 11	12	24 × 10
13	28 × 13	13	25 × 11
14	27 × 14	14	20 × 8
15	23 × 11	15	21 × 9
16	25 × 12	16	25 × 8
17	23 × 12	17	15 × 6
18	26 × 11	18	17 × 8
19	24 × 10	19	17 × 6
20	27 × 12	20	20 × 7
Average—	25 × 12	Average—	21.5 × 9

A study of the data tabulated above shows that the leaves of the diseased plants are 15 per cent shorter and 25 per cent narrower than those of the healthy plants. From these data it is estimated that ring spot caused 33 per cent injury to the affected plants in the reduction of the size of the leaf. In addition to the loss in size, the average number of leaves per plant was 2.25 less for the affected ones. The healthy plants averaged 14.75 leaves per plant and the diseased ones averaged only 12.5 per plant. This shows a 15 per cent reduction in the number of leaves per plant. Since ring spot caused 15 per cent reduction in the number of leaves per plant

and also 33 per cent reduction in the size of the leaves, there was a total loss of 43 per cent per plant caused by this disease. There was also considerable tissue injury to the leaves on the affected plants.

TOTAL LOSS FOR WASHINGTON COUNTY IN 1929

The total loss for Washington County was found by multiplying the average extent of injury to the plant by the percentage of plants affected. According to the records, 7.6 per cent of all the plants in the county were affected with ring spot, and the value of the affected plants was reduced 43 per cent. The total loss for the county, therefore, was 43 per cent of 7.6, or 3.26 per cent of the entire crop.

It is estimated that in 1929 2,500 acres of tobacco were grown in Washington County and that the average production was 1,050 pounds per acre. The average price paid for Burley tobacco on the Abingdon market was 32 cents per pound. Therefore, the total crop produced was 2,652,000 pounds and the loss due to ring spot about 85,575 pounds. This makes a loss of $27,384.00 to the farmers of Washington County as a result of the ring-spot disease, in 1929.

DISCUSSION OF RESULTS

The writer's observations in 11 of the leading tobacco counties of Virginia, representing all types of tobacco grown—Burley, flue-cured, fire-cured, and sun-cured—lead him to believe that the ring-spot virus is not any more virulent for one type or variety of tobacco than it is for another but that all types are about equally susceptible. It has been shown by Wingard[9] that the ring-spot virus in expressed juice does not remain virulent for more than 24 hours at ordinary temperatures. It does not seem possible, therefore, for the virus to be carried over in the plant-bed soil or old tobacco or other plant refuse.

As reported above, various plants and weeds were closely observed in an attempt to determine which were capable of harboring the ring-spot virus. Twenty-five different species of plants were sent to the State Agricultural Experiment Station for inoculation tests with tobacco. Sweet clover and stick weed were the only plants found to be naturally infected with the ring-spot virus.

It seems that some insect must be responsible for transmitting the ring-spot virus from affected weed hosts to tobacco; however, results were not obtained to support this hypothesis. Further study along this line under varying conditions is urgently needed.

The masking of the symptoms of this disease is shown to a very striking degree. Fields were observed in the middle of the growing season to

[9] Loc. cit.: See footnote 5.

be badly affected with ring spot and, in one case, as much as 90 per cent infection was found earlier in the season. Later in the season, about topping time, the symptoms became masked to such a degree that it was difficult to locate them and, finally, many disappeared altogether. The field that showed 90 per cent infection on July 3 showed only 35 per cent infection on August 3. In another field, where counts were made on July 3, 75 per cent infection was noted. On August 3, the infection had become masked to such an extent that only 30 per cent of the plants showed symptoms of ring spot. A similar change was seen in practically every case. The masking of the ring-spot symptoms is very interesting. It occurs both in the field and under greenhouse conditions.

The extent of injury to plants was rather difficult to determine because, even though the plant may have had the disease and shown typical markings early in the season, at topping time and later, when these studies were made, the symptoms in many cases had completely disappeared. It could not be definitely determined whether certain plants were dwarfed as a result of ring spot or because of some nutritional disturbance. However, measurements were obtained which give a fairly accurate idea of the injury caused by the disease. There may be considerable injury not noticeable in the weight and quality of the leaf; and, consequently, the injury due to stunting may be greater than the writer's studies actually indicate.

For 2 consecutive years the sales of ring-spot-affected tobacco have been closely observed on the warehouse floors. Due to the masking of symptoms previous to cutting, it was very difficult to find any evidence of the ring-spot disease in the cured leaves, known to be severely affected during the growing season. There apparently was no reduction in the price paid for such affected tobacco, although there undoubtedly was a considerable reduction in the weight of leaves. Therefore, the total receipts for the crop were considerably less. If the tobacco had been sold in the field before cutting, the buyer most probably would have cut the price on the affected tobacco to a considerable extent, owing to the very conspicuous nature of the ring-spot symptoms.

SUMMARY

1. Steam sterilization of tobacco-plant beds did not prevent ring-spot infection in the fields planted from these beds.

2. Negative results were obtained in all cases in attempts to transmit ring-spot virus by means of tobacco flea beetle, cucumber flea beetle, leaf hopper, aphis, firefly, and the tobacco horn worm.

3. Stick weed, *Verbesina alternifolia,* and sweet clover, *Melilotus alba,* were found naturally infected with ring spot. Infection was readily obtained on tobacco with the expressed juice from these plants.

4. Twenty-five other species of weeds were tested for ring spot, with negative results.

5. The percentage of ring-spot infection in 10 counties in Virginia in 1927 was 2.5 per cent. In Washington County, in 1928, it was 3 per cent and, in 1929, 7.6 per cent.

6. There was an average injury of 43 per cent to the affected plants.

7. It is estimated that ring spot caused a total loss of $27,384.00 in Washington County, in 1929.



THE RELATION OF CANKER TREATMENT TO FIRE-BLIGHT CONTROL

J. A. McCLINTOCK

It has long been accepted that blossom-visiting insects play an important part in disseminating the fire-blight organism, *Bacillus amylovorus* (Burr.) Trev. Recently, however, the work of Miller (2) and Tullis (5) indicates that this organism can be spread by rain without the aid of insects. An example of this type of dissemination is illustrated by figure 1, in which the upper apple branch was naturally infected with blight in 1929. In the spring of 1930 bacteria escaped from this 1929 infection and were washed by rain to the new growth on lower limbs, where they subsequently infected both leaves and shoot tips. Numerous cases of this type of infection from hold-over cankers were observed in 1930. Such infections indicate the importance of meteoric water in spreading blight bacteria from old bark lesions to new growth nearer the ground. But meteoric water alone could not account for the epiphytotics of blight, especially blossom blight, which have occurred in Tennessee during the past 2 years. Therefore, it must be concluded that blossom-visiting insects are still the most important factor in wide dissemination of fire-blight bacteria.

The work of Rosen (3) gives further evidence that bees are a factor in harboring and spreading the fire-blight organism. The findings of Thomas (4) offer a concrete explanation of the fact that a few pear trees with their blossoms blighted early in the spring serve as centers for infesting bees which spread the bacteria to entire apple orchards when these bloom some weeks later. Even though he succeeded in obtaining virulent blight bacteria from the surface of honeycomb after 55 days, Thomas (4) expresses doubt that such bacteria would have lived over winter in that way. The conclusion is that hold-over cankers are one of the most important known means by which *Bacillus amylovorus* lives from fall to spring.

It is generally agreed that the fire-blight organism may overwinter in both pear and apple cankers. Of the apple varieties, Transcendent crab is one of the most favored hosts for hibernation. Their ability to overwinter the blight bacteria, coupled with their early spring blooming, makes Transcendent-crab trees second only to Kieffer pears as important centers for bee contamination. In the Southern States the Transcendent crab seldom occurs in large numbers, but it is not uncommon to find one or more trees of this variety in home orchards. Since it is prized for jelly making, owners hesitate to destroy their trees to reduce blight of surrounding varieties. In studies on blight control it was therefore important to seek a practical method that did not involve the cutting down of any trees.

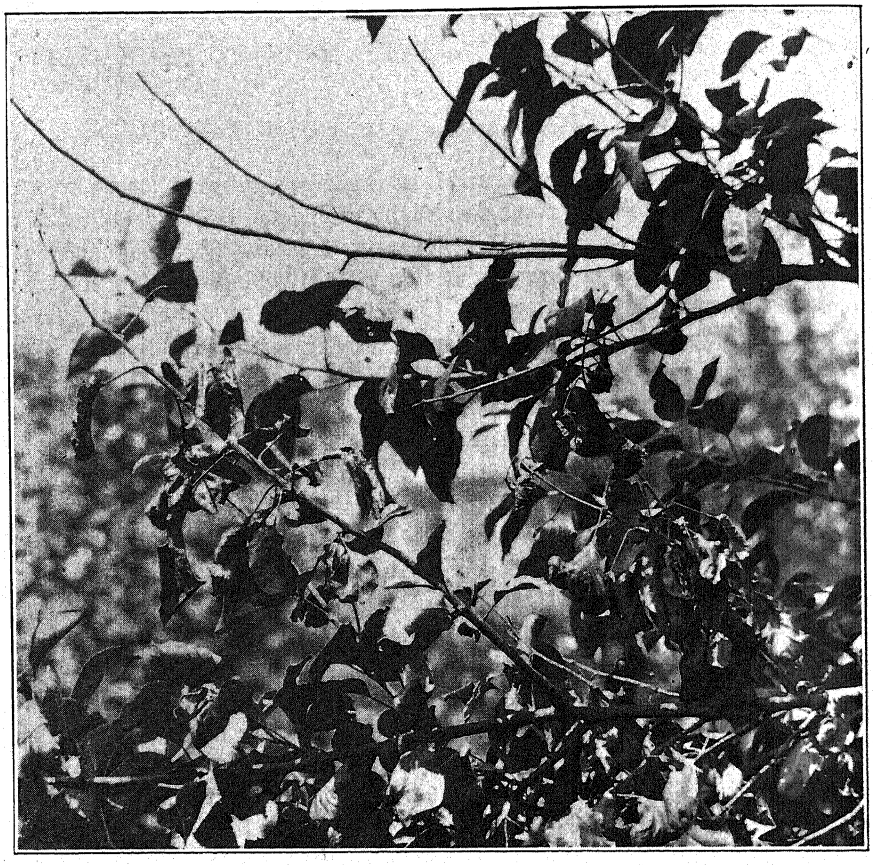


FIG. 1. The bare upper limbs were killed by natural blight infection in 1929 and left without treatment. In the spring of 1930 rain carried bacteria from the 1929 infection, causing a number of fresh infections on the new growth of the lower limbs.

Efforts to control fire blight by surgical methods have not been sufficiently successful to convince the average grower that this is a practical method. Failure to get results from surgical methods alone led the writer to turn to the zinc chloride treatment by which Day (1) in California had succeeded in killing blight bacteria in pear cankers. During the past 2 years this treatment has been applied to hundreds of apple and pear cankers of many varieties, but the experiments here reported were confined to the Transcendent-crab variety. Among others treated in 1929 were 3 bearing trees of this variety growing in a test orchard on the University Farm. One of these trees is a standard, the second a semidwarf, and the third a true dwarf, figure 2. These trees had been in bearing for several

years and, regardless of their rootstock, had bloomed, foliated, and set and matured fruit at approximately the same time. During the heavy blight epiphytotic of 1929 all 3 trees became infected. This resulted in the loss of considerable fruit and in the development of numerous blight cankers. Because it was desired to kill the blight bacteria in these cankers regardless of other effects, the strongest solution, 53 per cent, recommended by Day (1) was used in treating all cankers. The solution was painted over all the infected areas and for a distance of 8 to 12 inches beyond external symptoms of blight. The first treatments were marked with dated tags, and the few subsequent infections were similarly treated and labeled.

In the early-season infections it was obvious that the bacteria were spreading rapidly into new tissues prior to treatment, but such advance appeared to cease shortly after the zinc chloride solution was applied. In



FIG. 2. The dwarf Transcendent-crab tree as it appeared during the summer of 1926. It had borne a crop of fruit each year since 1926 but was heavily infected with blight in 1929.

most cases the treatments were made as soon as external symptoms of blight appeared; therefore, a few deep cankers developed. In a few cases, however, the organism entering through short fruit spurs induced deep cankers involving the cambium. The zinc-chloride solution appeared to penetrate these and kill the bacteria the same as in the shallow cankers. By labeling all infections after treatment the writer was sure that these 3 Transcendent-crab trees entered the dormant priod in the fall of 1929 with no untreated cankers.

In order to eliminate insect transmission as far as possible, these 3 trees, while still dormant, were on March 7, 1930, enclosed in wood-frame cages, covered with 16-mesh galvanized-wire screen. The bases of the cages were enclosed with boards set in the ground so as to exclude entrance from that source, and the doors were kept closed except when the writer was entering or leaving a cage.

Although the cages afforded the desired protection from insect infections, yet from the observations that follow it is seen that the results were adverse in that exclusion of insects prevented fruit setting. These Transcendent-crab trees, as usual, bloomed considerably in advance of all other apple varieties; their blossoms were fully open by April 1, 1930. Kieffer pears were in bloom about March 20, 1930, and showed well-developed cases of blossom blight by the time Transcendent-crab trees were in bloom. When the caged crab trees bloomed many blossom-visiting insects were attracted, but very few gained entrance through the 16-mesh screen, and the flowers remained practically free from flying insects throughout the blooming period. The blossoms appeared normal, the pollen was abundant, and the weather bright and favorable for pollination, yet nearly all dropped without setting fruit. During this period no blight developed on any of the 3 caged trees. This indicated that no blight organisms had overwintered in the treated cankers formed on these trees the previous season and that no blight-contaminated insects had reached the blossoms. Neighboring uncaged trees of standard varieties bloomed about April 18, 1930, and blossom and twig blight was abundant on apple trees throughout east Tennessee, but no sypmtoms of infection had developed on any of the other caged trees.

It is now accepted that blossoms are not the only plant parts through which blight bacteria enter the host and that water, as well as insects, plays an important part in bacterial spread. In order to verify these findings under controlled conditions the following experiment was conducted. On May 6, the writer cut from young, bearing apple trees of a number of varieties sufficient blighted fruit spurs and young shoots to fill a 16-quart berry carrier about 7 x 11 x 22 inches inside measurement. During a shower late in the afternoon of the same day the container of blighted twigs was placed

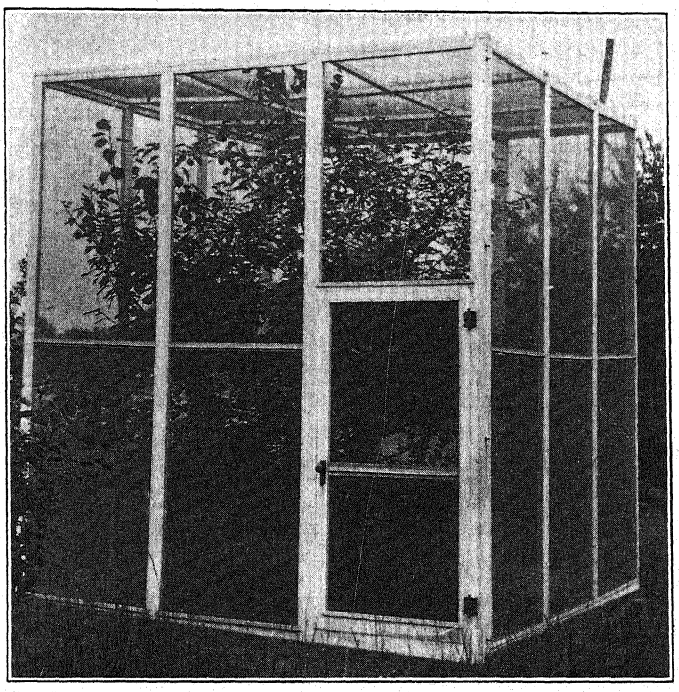


FIG. 3. The standard Transcendent-crab tree which had been in bearing for several years and which was heavily infected with blight in 1929. All infections were treated with 53 per cent zinc chloride solution in 1929, and the tree was enclosed in the cage March 7, 1930, while still dormant. No blight developed on this tree in 1930.

on the screen covering the top of the cage over the semidwarf tree. Cloudy weather with showers occurred on May 7, 8, and 9, followed by a heavy rain on the 10th and rain on the 13th, 16th, and 17th. On June 7, 1930, 3 well-developed cases of twig blight were observed on branches of the caged tree just under the carrier of blighted twigs. No blight had developed on the caged standard tree (Fig. 3), which served as a check; therefore, the blighted twigs on the semidwarf tree were undoubtedly due to bacteria carried to them from the container of blighted fruit spurs and shoots by meteoric water. After removing the carrier from the top of the cage the writer entered, cut the 3 blighted twigs from this tree and disinfected the cut surfaces. The blighted twigs were placed at once in a moist chamber and 2 days later all 3 exhibited drops of bacterial ooze. This ooze was found, by infection experiments, to contain the fire-blight pathogene.

It is generally considered that slowly growing trees are less susceptible to blight, but results obtained with the dwarf Transcendent-crab tree under controlled conditions indicate that this is not always the case. For

on May 12, 1930, blighted blossom spurs and new shoots were cut from a Bartlett-pear tree in the Station plots and placed on the cage covering the dwarf Transcendent-crab tree. No bacterial ooze was observed on these blighted pear shoots at the time they were cut, but the rains of May 13, 16, and 17 must have washed bacteria from them to growing tips of the caged dwarfed tree, for, subsequently, 2 twigs developed typical blight. The caged standard tree developed no symptoms of blight throughout the season; therefore, the blight infections on the dwarf tree must have come from bacteria from the blighted pear twigs on top of the cage. After removal of the blighted tips and disinfection of the cut surfaces no further blight developed on the 2-caged trees.

With blossom-visiting insects excluded from these caged trees, the absence of blight infection during and immediately following the blooming period is proof of the effectiveness of the 53 per cent zinc chloride solution in destroying blight bacteria in hold-over cankers on these apple trees. The fact that 2 of these caged trees were later artificially infected through water-borne bacteria, while the unexposed tree remained healthy, indicates that these trees were equally susceptible to blight in 1930 as when naturally infected in 1929.

In this and other experiments with apples and pears no serious injury to limbs has resulted from treatments of blight cankers with 53 per cent zinc chloride solution. Therefore, these data indicate that much could be done to reduce the primary spread of the blight organism and the spring contamination of bees if the hold-over cankers were given a thorough application of zinc chloride.

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BACTERIAL LEAF SPOT OF VIBURNUM

H. H. Thornberry and H. W. Anderson

Bacterial leaf spot of Viburnum was first observed by the writers on May 25, 1929, on the University of Illinois campus. Further investigations of the Virburnum plantings on the campus revealed infection on certain species only.

SYMPTOMS

Infections are for the most part on the leaves, but young stems are somewhat susceptible. Lesions on the fruits or in the inflorescence were not observed. Leaf lesions are more conspicuous than those on the stems.

The early stage of leaf infection becomes manifest in water-soaked, circular areas. In about 4 days after these areas are visible they develop into irregular, shrunken, brown spots from 2 to 4 mm. in diameter (Fig. 1). The center of aged lesions appears somewhat transparent. Microscopic examination of stained sections of such lesions shows disintegration of the mesophyll cells.

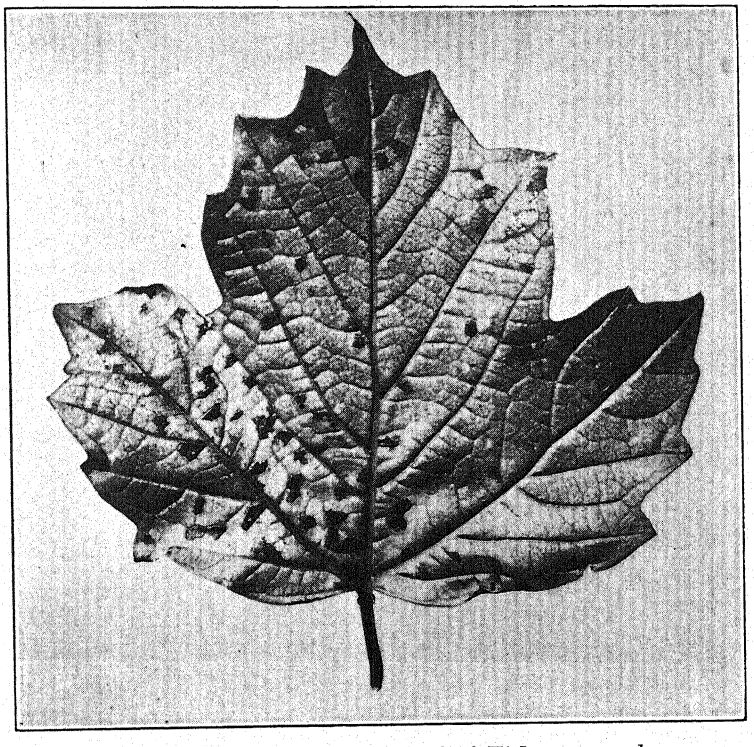


Fig. 1. Natural infection on a leaf of *Virburnum opulus*.

Young lesions of the stem seldom become conspicuous. At first they are water-soaked, elongated, and superficial. Later, they develop into slightly shrunken streaks, limited to the cortical regions of the stem (Fig. 2).

Microscopic examination of affected leaf tissues shows abundance of bacterial ooze from the margin of the lesion, but the shrunken, transparent center of older lesions is free from masses of bacteria. Diseased tissues of stems contain abundance of bacteria which ooze from sections in water.

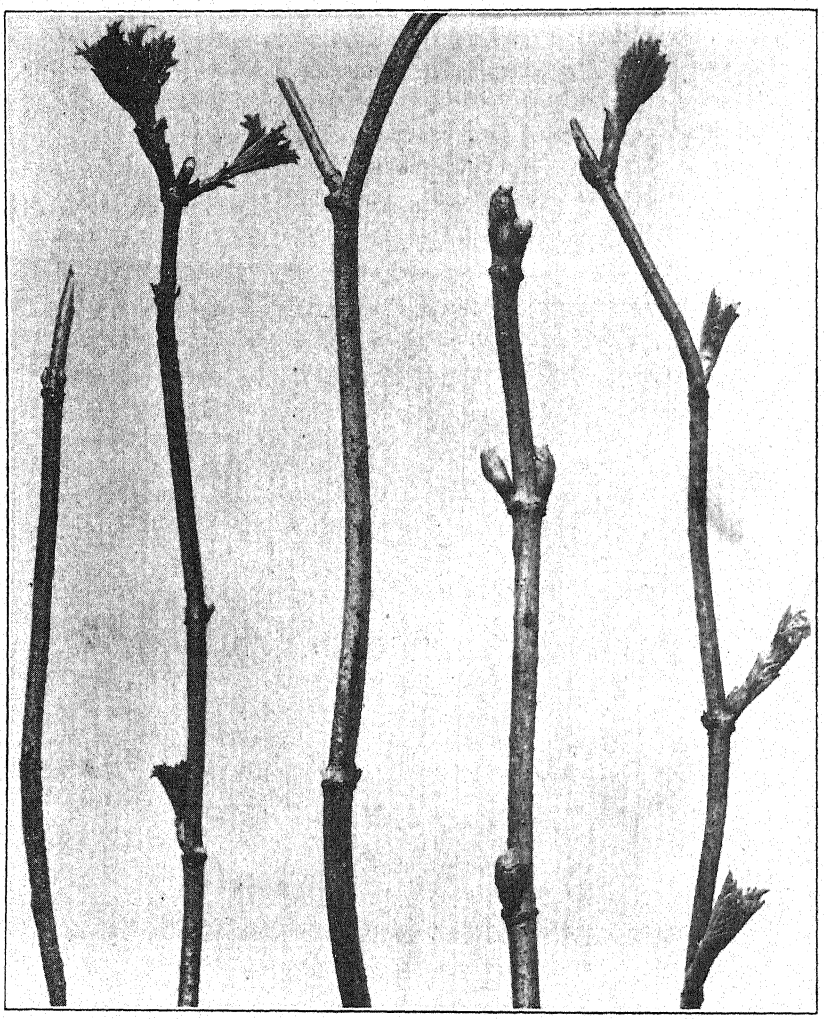


FIG. 2. Stem lesions in which bacteria live over winter. The lesions appearing as brown shrunken streaks are limited to the cortex.

DISTRIBUTION AND HOST RANGE

The disease has been observed on *Viburnum opulus* L., *V. tomentosum* Thunb., and *V. dentatum* L. on the University of Illinois campus. Other species on the campus, *V. acrifolium, V. americanum* Mill., *V. Carlesii* Hemsl., *V. cassinoides* L., *V. lantana* L., *V. lentago* L., *V. molle* Michx., *V. prunifolium* L., *V. Sargentii* Koehne and *V. Sieboldii* Miq., were not infected. Viburnums investigated at other points in the State, including Champaign and Urbana, were free from infection. The disease has not been observed elsewhere and no reference to it has been found in the literature. Since the disease is easily noticeable when in a severe form, it would seem that it could hardly have been overlooked had it been common or widely distributed. A limited number of inspections have been made on wild species of Viburnum and in nurseries without revealing any further distribution.

ISOLATION

The dilution-poured-plate method was used to isolate the causal organism. Small sectors from lesions were placed in a drop of sterile water on a microscopic slide and then examined with low-power magnification. The sectors which showed bacterial ooze were transferred to 1 cc. of sterile water in a sterile Petri dish and allowed to remain 30 seconds or longer, depending on the amount of observed bacterial ooze. By transplanting one loopful of the inoculum to a second Petri dish containing 1 cc. of sterile water, and from the second to the third dish, suitable dilutions were obtained. The plates were poured with melted dextrose agar that had been cooled to 40° C. Typical colonies developed within 4 days when incubated at 25° C. Stock cultures were obtained by transferring from single colonies to dextrose-agar slants.

The organism was reisolated from artificially produced lesions.

INOCULATION

Stomatal entrance was readily demonstrated by atomizing a suspension of the pathogene on young leaves of *Viburnum opulus*. Inoculations thus made on June 2, 1929, during a gentle rain, developed typical lesions within 9 days. Inoculations again made on June 12 resulted in evident infection by June 25. It was observed that rubbing the leaves between the thumb and fingers or scratching them with a sharp needle, while the suspension was atomized, aided entrance (Fig. 3).

OVERWINTERING OF THE PATHOGENE

The pathogene lives through the winter in the tissues of dormant buds and in small cankers on young stems. Primary leaf infection in the spring results for the most part from the inoculum in infected tissues near buds

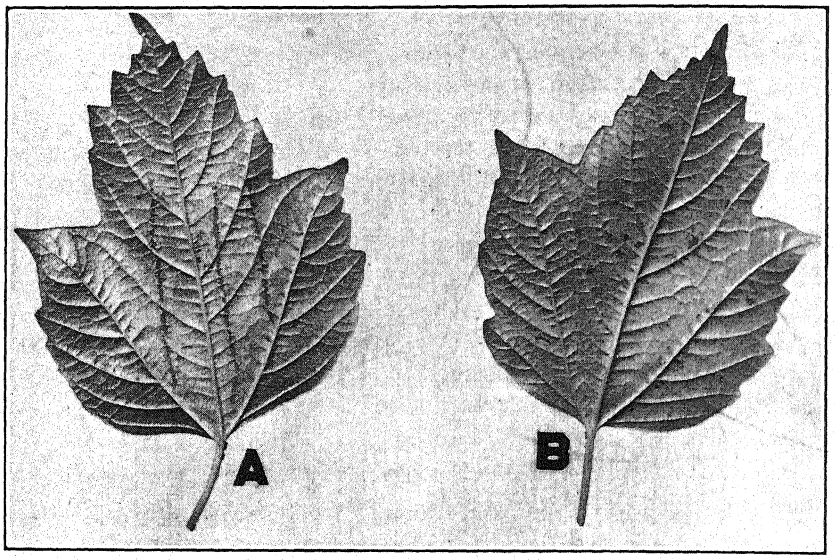


FIG. 3. Artificial infections. A. Inoculation made by atomizing a suspension of the pathogene and by scratching the leaf with a sharp needle. B. Inoculations made by atomizing the suspension without injuring the leaf.

and not from the stem cankers. Bud and stem infections occur during late summer and early fall, before infected leaves drop and while the young stems are succulent. The pathogene probably exists in a semi-active condition in the diseased tissue during the winter months.

Infected buds, which were first observed on April 20, 1930, contained visible lesions in the region of the cortex of the stem adjoining these buds. Stained microtome sections of the bud and adjoining tissue showed bacteria concentrated in definite areas of disintegrated cells. Serial sections showed that infection originated in the stem at the axis of the bud and advanced through the cortex to the basal tissues of the bud. Infection probably occurred during early fall before the infected leaves dropped. Invasion continued for the most part during fall and the organism remained semi-active during the winter months. During spring the pathogene became active and the organism advanced farther into the outer portion of the bud and adjoining tissues. Buds that were severely infected were killed outright.

The temperatures through September and October and April and May were favorable for continuous growth of the organism. Furthermore, meteorological data show that the temperature on several days during each of the winter months was above 12° C., the limit for growth of the organism in culture; thus it was possible for the bacteria to develop during these periods.

Primary leaf infections were evident April 28, 1930, 1 week after buds opened. Since the incubation period is 7 to 9 days, natural infection must have occurred when the buds expanded. The severity of local infections on leaves developed from certain buds (Fig. 4), and the absence of general infection indicates that the infected buds were the source of inoculum. Local infections in all cases were associated with stem lesions near the original position of the bud.

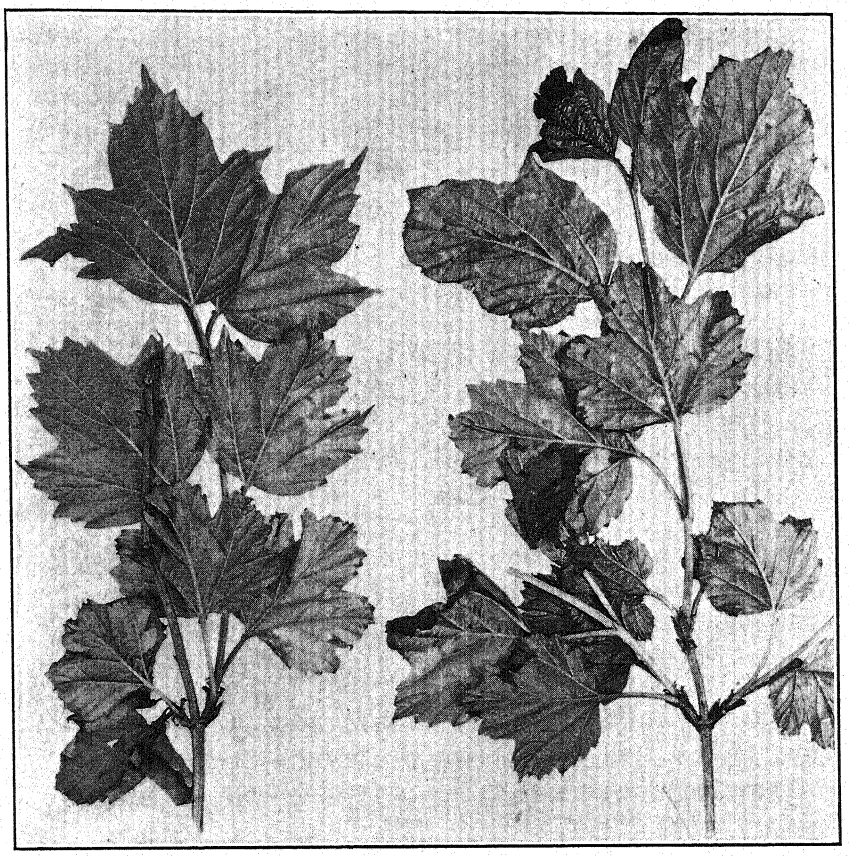


FIG. 4. Primary spring infections, traced to lesions under the bark of the terminal portion of the stem.

The pathogene remained viable through the winter months in cankers, but in no case were primary infections traceable to them. This inoculum, as well as that from buds killed from severe infection and from overwintering bacteria in the infested leaf débris in the soil, no doubt is capable of producing infection, but not until the leaves are evolved. By this time primary infections from buds are well established.

DESCRIPTION

Phytomonas viburni n. sp. is a short rod 1 to 2 microns long by 0.5 to 1 micron wide, motile by means of 2 or 4 polar flagella, occurring singly, in pairs, and in short chains, but mostly in pairs. It is capsulate but forms no spores; is gram-positive and not acid-fast; forms round, entire, pale dull gray colonies on dextrose beef-extract agar; clouds bouillon and forms a pellicle; has no diastatic action; does not liquefy gelatin; does not ferment xylose, rhamnose, glucose, mannose, galactose, fructose, lactose, maltose, sucrose, rhaminose, raffinose, dextrin, inulin, glycerol, mannitol, sorbitol, dulcitol, nor salicin; does not reduce nitrates; produces an alkaline reaction and causes neither coagulation nor peptonization in litmus milk without reduction of litmus; produces neither hydrogen sulphide nor indol; is aërobic; optimum, minimum, and maximum pH for growth are 8.5, 4.8, and 10.4, respectively; optimum, minimum, and maximum temperatures for growth are 25°, 12°, and 35° C., respectively; causes brown irregular spots on leaves and elongate lesions on petioles, young shoots, and twigs of species of arrowwood, *Viburnum opulus, V. tomentosum,* and *V. dentatum.*

A culture of *Phytomonas viburni* has been sent to the American Type Culture Collection, John McCormick Institute of Infectious Diseases, Chicago, Illinois.

SUMMARY

1. Bacterial spot of Viburnum was discovered May 25, 1929, on the University of Illinois campus.

2. The disease is apparently of local distribution.

3. The pathogene lives over winter in cankers, in infected buds, and possibly in infested leaf débris in the soil. Primary infection in all cases observed developed from infected buds.

4. Artificial inoculations produced typical lesions and the organism was reisolated in pure culture.

5. A description is given of *Phytomonas viburni,* the causal organism.

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DIDYMOSPHAERIA OREGONENSIS, A NEW CANKER ORGANISM ON ALDER[1]

L. N. Goodding

INTRODUCTION

Field studies on white-pine blister rust in the Pacific Northwest have led to some observations on other fungi affecting forest trees. In 1929 the writer noticed a peculiar trunk canker on alder (*Alnus rubra* Bongard). At first glance it appeared to be the result of insect work or injury due to sapsuckers. Closer examination, however, proved it to be a well-defined canker having a fungus constantly associated with it.

A study of the fungus has shown that it is an undescribed Didymosphaeria. L. E. Wehmeyer, of the University of Michigan, in a letter dated April 26, 1930, suggested that the most closely related Didymosphaeria previously described is *D. nana* var. *brachyspora* Sacc.[2] This occurs on the leaves of an Alaskan alder. Since, however, the species here discussed is found only on the trunks and limbs of the alder and is obviously different from one other close affinity in the genus, the writer proposes the name **Didymosphaeria oregonensis** n. sp.

DISTRIBUTION

This fungus is general throughout western Oregon. Two specimens were collected in northeastern Oregon, one in western Washington, and two in Idaho. It probably is common throughout the Northwest. This fungus has been found on three species of alder; namely, *Alnus rubra, A. tenuifolia* Nutt., and *A. sinuata* (Regel) Rydb. It is worth noting that abundant *Betula glandulosa* Michx. associated with alder sustaining a heavy infection of *Didymosphaeria oregonensis* at Horse Thief Meadows, Hood River County, Oregon, showed no signs of infection.

ECONOMIC IMPORTANCE

The constant association of this organism with the marked banding effect and at times with the pronounced swollen cankers on the boles and branches of alder indicates that it is an active parasite. There seems to be some indication from observations in the Mud Creek region in the Mt. Hood National Forest that it becomes sufficiently active at times to kill the affected branches. This organism, however, has not been shown to be the cause of this killing. In many localities a mere band of roughened bark is

[1] This has previously been mentioned in a brief abstract in Phytopath. 20: 854. 1930.

[2] Sacc. Syll. Fung. 17: 679. 1905. (See also Harriman Alaska Expedition 5: 30. 1900.)

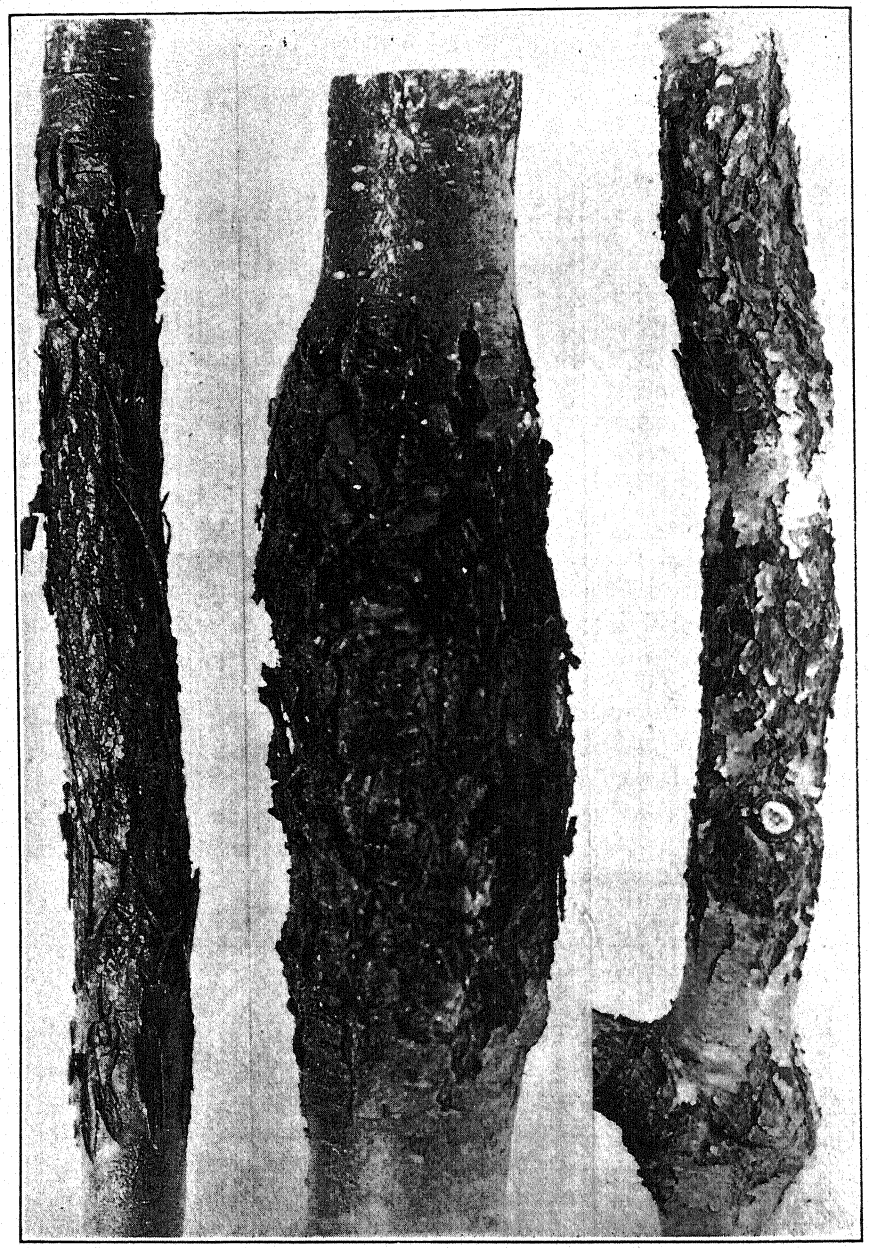


FIG. 1. Photograph of cankers on *Alnus rubra* caused by *Didymosphaeria oregonensis* Goodding. Natural size.

produced, but in others, or on certain trees, each canker forms a pronounced swelling. In no place where it has been observed can it be considered a serious pest, though in some localities young trees are deformed and stunted by very numerous cankers.

DESCRIPTION OF CANKER AND FUNGUS

Cankers with which the fungus is associated form bands around the trunks and limbs of the alder (Fig. 1). These bands vary from ½ inch to 2 feet or more in length. The organism apparently confines its activity to young limbs and trunks, ceasing to grow after the bark has become hard and thick. The scars, however, are often evident on the trunks of mature trees. While working on the tender bark the organism produces a marked zoning which readily reveals the age of simple cankers. A longitudinal section of a canker where swelling has taken place shows increased thickness of the annual layers following infection. This apparently is due to the release of tension where the fungus breaks the outer bark. Perithecia are produced only on the portion of the bark recently invaded. In the very early spring a slight discoloration of the bark beyond the band bearing the perithecia of the last season can be clearly discerned. By early May this reveals the growing perithecia by a pronounced pimpling. By midsummer these are producing mature ascospores. Perithecia are numerous, single or occasionally somewhat caespitose, those of the current season confined to a zone from a few millimeters to several centimeters wide. If this consti-

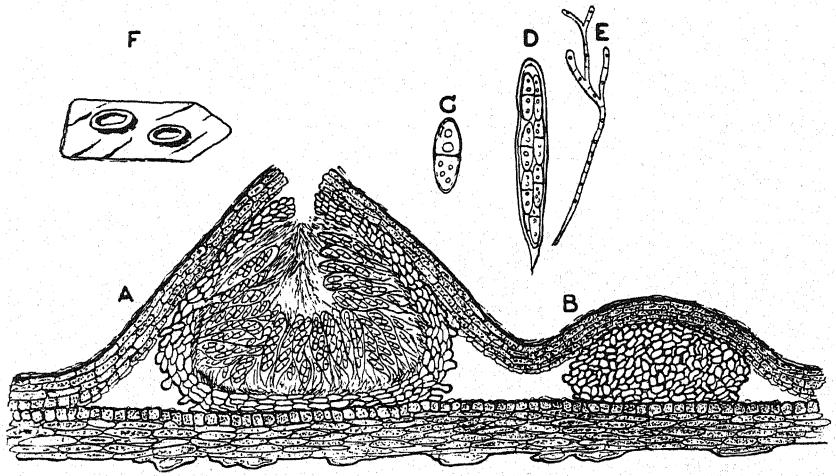


FIG. 2. A. A single perithecium. ×30. B. Wall of single perithecium. ×30. (A and B show the outer cortical layer raised by the perithecia.) C. A single ascospore. ×400. D. An ascus with spores. ×275. E. A paraphysis. ×275. F. A scrap of inner bark showing collapsed perithecia. ×5.

tutes the initial growth it starts as a narrow lens-shape area but soon forms a complete single band about the trunk or branch. As the perithecia reach maturity the outer cork layer of the bark is broken loose and raised. Finally, the outer layer of the bark exfoliates, leaving a roughened and ragged surface. The perithecia usually remain attached to the bark on the trunk or branch but occasionally peel off with the outer cork layer. After the contents of a perithecium are exuded, the upper portion collapses and the base forms a minute cup.

The technical diagnosis follows:

Didymosphaeria oregonensis n. sp.

Perithecia (Fig. 2) about 1 mm. across, globose, dark brown or black, opening by a very minute pore which does not protrude beyond the surface of the bark; the cavity is filled completely with the asci and filiform, branched, septate paraphyses; *asci* numerous, produced from the sides and bottom of the perithecia, cylindrical to clavate or quite irregular, 75–90 μ long, opening by a wide pore at the tip which does not turn blue with iodine, contain 8 spores; *ascospores* "kildare green" (Ridgway), rounded at both ends, 1-septate, slightly if at all constricted at the septum, 18–21 × 7–9 μ.

This organism apparently is related to *Didymosphaeria Wallrothii* (Hipp.) Sacc. & Trott.[3] from which it differs in having larger spores, less conspicuous perithecia, and nonprotruding ostioles. *D. Wallrothii* occurs on birch bark and has not been reported from North America. As suggested by Wehmeyer, *D. oregonensis* may also be related to *D. nana* var. *brachyspora* Sacc. In *D. oregonensis,* however, the spores are relatively much broader than those in *D. nana* var. *brachyspora.* This and the fact that *D. oregonensis* has never been reported as occurring on the leaves indicate that it is a distinct species.

SPECIMENS EXAMINED

On *Alnus rubra* Bongard

Oregon: Clackamas County, Still Creek, *L. N. Goodding,* May 30, 1929, 4961,[4] July 10, 1929, 4963, May 23, 1930, 5533, *L. N. Goodding* and *A. L. Hinckley,* July 4, 1930, 5534 and 5535; Mud Creek, *L. N. Goodding* and *G. D. Darker,* July 12, 1929, 4964; Yokum Falls, *L. N. Goodding* and *G. D. Darker,* July 15, 1929, 4965, *TYPE;* Crater Lake, *L. N. Goodding* and *M. C. Riley,* August 4, 1929, 4966; Colla-

[3] Sacc. Syll. Fung. 1: 715. 1882. 22: 174. 1913.

[4] All numbers refer to the Oregon State College Herbarium.

wash River, *L. N. Goodding* and *M. C. Riley,* August 16, 1929, 4968; Camp Creek, *L. N. Goodding* and *M. C. Riley,* October 13, 1929, 4967; Zig Zag River, *L. N. Goodding* and *M. C. Riley,* October 15, 1929, 4972; Rhododendron, *L. N. Goodding,* March 5, 1930, 4977, April 18, 1930, 5531, *L. N. Goodding* and *E. W. Lyle,* October 2, 1930, 5532; Columbia County, Vernonia, *L. N. Goodding,* March 19, 1930, 4978; Douglas County, Gunter Road, *L. N. Goodding,* November 23, 1929, 4975; Florence, *L. N. Goodding,* November 25, 1929, 4976; Canyonville, *L. N. Goodding,* February 22, 1930, 5527; Hood River County, Eagle Creek, *H. N. Putnam* and *L. N. Goodding,* October 18, 1929, 4973, *E. L. Joy* and *L. N. Goodding,* May 13, 1930, 4981; Hood River, *L. N. Goodding,* June 4, 1930, 5538; Horsethief Meadows, *L. N. Goodding* and *A. L. Hinckley,* June 5, 1930, 5539; Jackson County, Woodruff Meadow, *F. P. Sipe* and *L. N. Goodding,* August 6, 1930, 5541; Lane County, Triangle Lake, *F. P. Sipe,* September 13, 1930, 5528; Marion County, Breitenbush River, *M. C. Riley,* August 20, 1929, 4969; Polk County, Rickreall Creek, *L. N. Goodding,* June 8, 1929, 4962; Salmon River, *L. N. Goodding* and *M. C. Riley,* September 19, 1929, 4970; Black Rock, *L. N. Goodding,* April 23, 1930, 4979; Tillamook County, Boulder Creek, *L. N. Goodding,* October 25, 1929, 4974; Wasco County, Clear Lake Creek, *L. N. Goodding* and *M. C. Riley,* October 12, 1929, 4971.

Washington: Pierce County, Kantz Creek, *L. N. Goodding* and *M. C. Riley,* May 4, 1930, 4980.

On *Alnus tenuifolia* Nutt.

Oregon: Clackamas County, Summit Ranger Station, *L. N. Goodding,* May 23, 1930, 5530; Deschutes County, Tumalo Creek, *L. N. Goodding,* October 7, 1930, 5529; Umatilla County, Pearson Ranger Station, *E. W. Lyle* and *A. L. Hinckley,* July 4, 1930, 5542; Union County, Jarbeau Creek, *E. W. Lyle* and *A. L. Hinckley,* July 21, 1930, 5540.

Idaho: Clearwater County, Three Bears Creek, *C. C. Strong,* July 9, 1930, 5543; Rhodes Creek, *E. L. Joy,* September 5, 1930, 5544.

On *Alnus sinuata* (Regel) Rydb.

Oregon: Clackamas County, Mud Creek Trail, *G. D. Darker* and *L. N. Goodding,* July 11, 1929, 4982; Mud Creek, *L. N. Goodding* and *A. L. Hinckley,* June 4, 1930, 5536; Wapinitia, *L. N. Goodding,* June 4, 1930, 5537.

SUMMARY

This paper describes as new a species of Didymosphaeria found associated with a canker on three species of living alder in the Pacific Northwest. The name **Didymosphaeria oregonensis** Goodding is proposed. The known distribution of the new species is given as Oregon, Washington, and Idaho. There is also included a brief consideration of its relationship and economic importance.

OFFICE OF BLISTER RUST CONTROL,
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THE KELM MOUNTAIN BLISTER-RUST INFESTATION

WALTER H. SNELL[1]

During the past 15 years of intensive search for white-pine blister-rust infestations, momentary enthusiasm has given rise, in a large number of cases, to estimates of practically 100 per cent infection on various lots. In nearly all such infestations, the actual percentage of trees diseased has been found to be below 75 per cent and usually nearer to 50 per cent. There is at least one area (the only one known to the writer), however, in connection with which the original estimate was not extravagant. This Kelm Mountain lot is an outstanding example of blister-rust damage. Because of its inaccessibility, this plot is probably the least known to pathologists and foresters of all blister-rust plots; few have seen it and not many more have heard of it.

This area was discovered during a deer hunt by A. E. Fivaz and E. G. Woodward, of the Division of Blister Rust Control. Inability to find a single uninfected tree in the course of two casual inspections made the area of unusual interest from the point of view of blister-rust damage, and the writer established an experiment plot there in 1923. The plot is at the foot of Kelm Mountain, between the Chestertown and Horicon roads out of Warrensburg, in Warren County, New York. It can be reached only after a walk, mostly uphill, of about a mile and a half from either highway. The elevation of the lot is 1,200 feet. The infested area is in a sort of pocket in the mountains protected on one side by the steep wall of Kelm Mountain and near a small unnamed pond, not far from Kelm Pond. The lot is surrounded by mature pine and hardwood. In general, moisture conditions favorable for the propagation and development of fungous diseases seem to prevail. During the summer the undergrowth usually remains wet until nearly noon, fog is very common in the nights and early morning, the woods are dark, and the forest floor supports an unusually abundant mushroom growth.

The permanent plot consists of 2 acres, selected from an extended stand of white pine of uniform type. This plot, in its original condition, had 550 trees to the acre, divided quite sharply into 2 groups—one of open growth of 400 pines an acre and the other of about 3,000 trees an acre. In the dense part of the plot it appeared that, under normal conditions, an excellent stand would have been produced, since the competition had resulted in the development of well-spaced dominant trees with an undergrowth of badly suppressed trees that soon would have been eliminated by shading. The open part likewise promised a good stand. It is true that there were

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too few pines for this purpose, but the stand was so interspersed with hardwoods of the proper size as to provide the pines with the stimulus for height growth and the shading for clean boles.

At the time of the first study, in 1923, these pines were mostly 10 to 14 years of age. Those that now survive are therefore between 17 and 21 years old.

The open spaces in the stand are quite heavily covered with blackberry brambles. In 1923 there were 174 bushes of *Ribes rotundifolium* Michx., with a total leaf-bearing stem of nearly 9,000 feet in the open part of the plot. No bushes were found in the dense part of the plot nor in the surrounding mature-pine and hardwood growth. In 1923, 25 per cent of the leaf-bearing stems were dead and 50 per cent were dead in 1926. At the present time, the few surviving bushes are in very poor condition. They are, however, numerous enough to provide infective material for the new pine seedlings as they appear.

As far as can be determined, this land was cleared sometime prior to 1907, when the owner sold the property and moved away. This fact is corroborated by the age of the trees now on the plot.

In all, during the past 8 summers, 1,110 trees have been found on this plot. Of these, 96.5 per cent have been found infected at one time or another. There are at present only 1,006, or 90.6 per cent, with living cankers. The cankers on 64 trees (6 per cent) are now dead because of shading. About 9,000 cankers, in all, have been found on the plot, an average of about 9 per tree. One tree bore 298 cankers, and 7 trees had between 100 and 200. The cankers on the trees in 1924 were distributed as follows: 0.4 per cent on 1916 wood, 3 per cent on 1917 wood, 16 per cent on 1918 wood, 74 per cent on 1919 wood, 5.6 per cent on 1920 wood, and 1 per cent on 1921 wood.

There was not a dead tree on the lot in 1920. In 1923, 9 per cent were dead; in 1924, 14 per cent; in 1926, 30 per cent; in 1927, 42 per cent; in 1929, 60 per cent; and, in 1930, 69 per cent. There are still left on the plot 18 per cent of the entire number that will die in a few years, making an imminent mortality of 87 per cent of the entire stand.

There will be left on the lot 147 noninfected trees. Of these, however, 40 will not survive because of shading or other factors. This will leave only 107 living trees on the 2 acres. Most of these are severely suppressed.

There is at the present time no reproduction to make a new stand, nor have any seedlings matured since 1910. All of the young trees that presumably were seeded in between 1910 and 1920 were killed. We are certain that there must have been some reproduction in that decade, because there is reproduction on other lots not infested with the disease. The years 1911, 1913, 1915, 1917, and 1919 were good seed years in the Adirondacks.

There has been seeding in years since 1920, but most of these young trees are at present infected and are doomed to die. This plot is a good example of the 2-storey condition explained in former publications[2,3] except that, because of the continued severity of the disease, the lower storey (trees that seeded in since 1920) is sparser here than in most places.

The original stand of 2 acres, one portion of pure pine and the other mixed with hardwoods, was such that, undisturbed by disease, it probably would have produced a minimum of 60,000 and probably nearer 70,000 board feet of high quality lumber. The rust will have left only 147 trees noninfected and, of these, shading will remove all but 107. The 107 trees that will survive rust and shade (presupposing that no more of these are attacked by the rust in future years) will be worth almost nothing and the damage because of blister rust will therefore be practically complete.

To one who has followed the conditions on this plot since the earliest studies, the change within the past few years is very striking. Up to 1926, to casual observation, the plot looked like a thriving young pine stand, practically pure pine in one part and mixed with hardwoods in the other. Few dead or dying trees were visible when the lot was viewed from the open. Whereas, at that time, the vigorous pines were visible even in the hardwoods, showing through and crowding above them, there is now little pine foliage to be seen. The single pines in the hardwoods are dead and dying, and, in the thicker portions, most of the pines are already dead. At the present time, it is most strikingly evident that the lot is no longer a pine stand but that it has, within the space of a few years, become a hardwood stand.

Another interesting feature of this stand is the 147 pines that have withstood the blister-rust attacks of the 1910–1920 decade, especially the severe wave of the latter part. These trees, found free from disease in 1923, are still intact. That they are constantly exposed to attack is shown by the diseased condition of all the young trees that have come up since 1919. These trees apparently are immune from the blister-rust fungus. It is going to be of considerable interest to find out how offspring from the seeds of the 107 staunch survivors react to *Cronartium ribicola* Fischer. It is hoped that such tests can be made.

ALBANY, NEW YORK.

[2] Snell, Walter H. Blister rust in the Adirondacks. Jour. For. 26: 472–486. 1928.

[3] Snell, Walter H. Some observations on the white pine blister rust in New York. Phytopath. 19: 269–283. 1929.



A WITCHES' BROOM OF OCEAN SPRAY (HOLODISCUS DISCOLOR)[1, 2]

S. M. ZELLER

In the spring of 1925 a resident of Corvallis, Oregon, observed in his garden a "freak" plant of ocean spray, *Holodiscus discolor* Max. He had secured the plant from an open woodland in Marion County, Oregon. Ocean spray is a beautiful rosaceous shrub, native to the Pacific Northwest. We have chosen to call the abnormal condition of this ornamental shrub a "witches' broom." Many diseased plants have been observed in the foothills of the western slope of the Cascade Mountains in Oregon from Wasco, Hood River, and Multnomah counties in the north through Clackamas, Marion, and Linn counties to the south. One specimen was brought in by L. N. Goodding from west of Olympia, Washington.

When this disease first makes its appearance on a plant the new lateral branches from an old stem are very slender and wirelike, with rather short internodes and small leaves. As a rule, there are 2 or 3 of these slender laterals from each node, while in healthy plants the laterals are more stalky and but 1 from each node. In the second or third year there is considerable multiplication of the laterals from each node on stems 2 or more years of age, and these laterals are much branched in contrast to the laterals of healthy plants.

New canes, which arise from or near the crown after the plants become affected, are short and give a stiff appearance, as illustrated (Fig. 1). In these canes the internodes are short; the main stems have little tendency to branch, so there are usually no blossom clusters. There are several buds at each node and these produce very short spindly laterals.

The leaves of affected plants are very small and crowded, giving the canes a very leafy appearance. Where they are not shaded they turn a bronzy red early in the summer. This general reddish tone may appear early in June when the spring is cool. This color stands out in contrast to the bright green of neighboring healthy plants.

Plants showing symptoms of the disease on 1 cane only were marked to determine subsequent development. As a rule, all of the aboveground portions of the plant showed symptoms the following year. All new canes from below or just above the ground in such cases showed advance symptoms of the disease.

In the early spring of 1927, buds from diseased plants were grafted into large healthy stems, 1 year old. In 6 out of 15 trials, the buds grew and

[1] Published with the approval of the Director as Technical Paper No. 136 of the Oregon Agricultural Experiment Station.

[2] An abstract of this paper appeared in Phytopath. 29: 851. 1930.

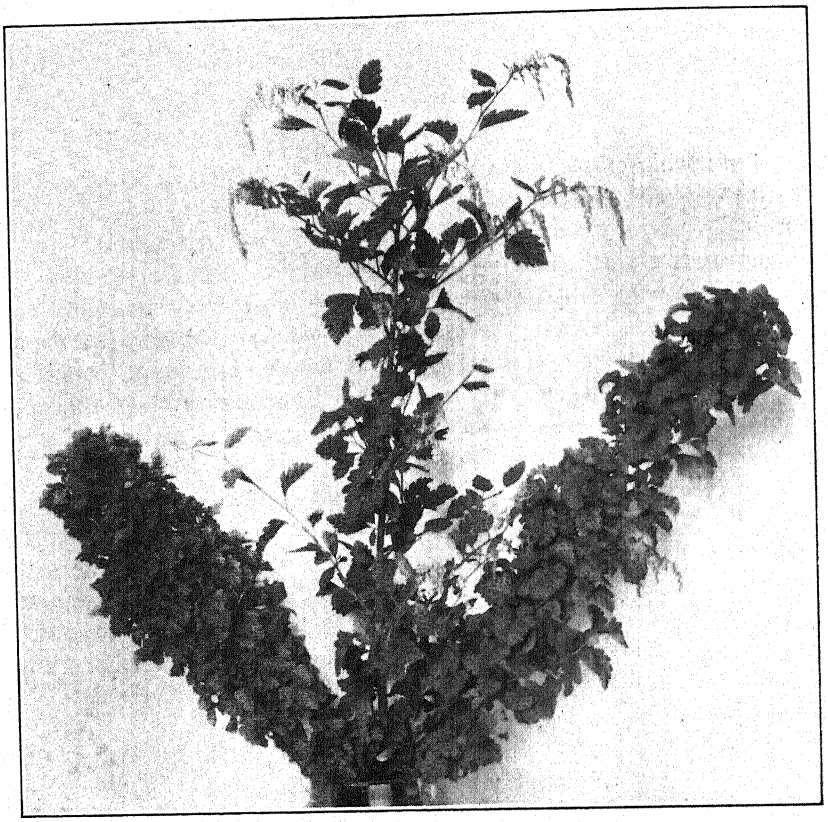


FIG. 1. Two branches of ocean spray affected with witches' broom. The middle upright branch is from a healthy plant.

in all 6 cases the disease was transmitted. The grafted node in such cases is the first to show symptoms. Then, the new growth appearing on the stock above the grafted bud shows a spindliness, but, during the remainder of the season, no symptoms are evidenced in other canes of the plant. In the second season (1928), however, in all 6 cases the whole plant showed evidence of the disease and the new canes were dwarfed with typical, advanced symptoms.

In an effort to discover the possibilities of insect transmission small laterals from diseased plants with aphis, *Aphis spiraeae* Schout., on the young leaves were placed in considerable quantity on 10 healthy plants on May 10, 1927. No symptoms of the disease made an appearance that season. In the spring of 1928, however, on 4 of these plants all of the new canes from near the ground and all of the lateral branches of any size showed rather advanced symptoms of the disease. On May 16, 1928,

a similar transfer of aphids to 12 healthy ocean-spray plants was repeated and, at the same time, aphids from diseased plants were transferred to 4 plants of *Spiraea thunbergii* Sieb., 2 plants of *S. Vanhoutei* Zabel, 1 plant of *S. prunifolia* Sieb and Zucc., 7 plants of *S. Douglasii* Hook., and 3 plants of *Physocarpus capitatus* (Pursh.) Ktze. In the summer of the next year, 1929, 9 of the 12 plants of ocean spray showed symptoms of witches' broom, but none of these other closely related rosaceous plants, to which aphis had been transferred, showed any indication of the disease. No disease resulted where aphids were transferred from healthy plants to 14 healthy plants. This work was done in the open field where adequate controlled conditions were not available, but the results appear sufficiently consistent to warrant notice. The possibility remains that there may be other means of transmission than the two suggested.

The tissues and buds of affected plants have been examined for a possible causal fungus or insect, such as mites or nematodes, but, so far, none has been found. The symptoms and performance of the abnormal condition thus far observed suggest that it might belong to the ever-increasing list of so-called virus diseases.

SUMMARY

A description is given of a disease which causes broominess and dwarfing of *Holodiscus discolor* (ocean spray), an attractive native shrub of western North America. The disease, called "witches' broom," has been found in the foothills of the western slope of the Cascade Mountains from Linn to Wasco counties in Oregon and in Thurston County, Washington. Budding of diseased nodes into healthy stems and the transfer of *Aphis spiraeae* from diseased to healthy plants have both apparently induced the disease. The symptoms and performance of the disorder suggest that it may be a virus disease.

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THE TOXICITY OF WATER-SOLUBLE EXTRACTIVES OF WESTERN YELLOW PINE TO LENZITES SEPIARIA[1]

BERNARD A. ANDERSON

INTRODUCTION

The following study of the toxicity to fungi of water-soluble substances, extracted from western yellow or Pondosa pine, *Pinus ponderosa* Lawson, and of volatile oils driven off during kiln drying, has been undertaken to furnish additional information on the durability of Pondosa pine.

Extraneous components of wood that include tannins, resins, dyes, gums, oils, alkaloids, etc., are chemical products of the tree. These components may be found in some woods and absent in others. They are usually present in the xylem of the tree and can be extracted by suitable solvents. According to Hawley and Wise (4), these substances are not considered an integral part of the cell wall.

At the present time research work is being carried on in the Forest Research Laboratory at the University of Idaho on various factors influencing the decay of wood. This experimental work is part of a program for the determination of the uses to which Inland Empire woods are especially adapted.

It is well known that in nearly all commercial species the durability of timber varies with the amount of heartwood and sapwood (7). Heartwood is generally more durable than sapwood (5). Accurate information covering the relative durability of heartwood and sapwood of various species, however, is comparatively scarce.

Pondosa pine may contain from 2 to 12 inches of sapwood, varying with the maturity of the tree. Service records prove that Pondosa pine heartwood is more durable than the sapwood. Is, then, the difference in durability of heartwood and sapwood due to the presence of sugars, protoplasm, or starches in the sapwood? Is it due to the presence of resin or oils or to the deposition of certain substances in the heartwood? Or, is it due to the presence of water-soluble toxic substances in the heartwood not found in the sapwood? These questions are as yet not answered and an attempt to

[1] Presented as a thesis for the Master's Degree in Forestry. School of Forestry, University of Idaho.

gather these data is here recorded. It is principally with the water-soluble substances that this paper deals.

Rose and Lisse (11) in their work on the chemistry of wood decay in 1917, found that the amount of hot- and cold-water extractives secured from wood varied with the stage of decay. In percentage relationship of extract to wood there was a decrease of cold-water extract from 4.03 per cent in sound Douglas fir heartwood to 1.16 per cent; in hot-water extracts, an increase of from 2.23 per cent to 7.77 per cent. The results of the cold-water extraction probably are not reliable because, as Rose and Lisse explain, "quite evidently the first of these could hardly be reliable because with increasing solubility in cold water there would be an increasing tendency towards loss by leaching out." The results would undoubtedly vary with the type of rot organism present in the wood, since fungi seem to vary in the manner in which they attack the components of wood (5).

A large number of heartwoods that are durable and resistant to decay under actual service conditions have been shown to contain water-soluble extractives in large amounts (3). On the other hand, hardwoods readily attacked by decay are usually deficient in such extractives. The kind of extractive varies with the species. In chestnut, black locust, oak, and red mulberry tannin exists; in Osage orange and black walnut, a soluble coloring matter is found in large quantities; redwood possesses an unknown coloring matter (3); western red cedar also contains a highly toxic brownish extractive (13). From such nondurable woods as birch, maple, or red alder, only indefinite and colorless extractives have been secured (3).

In the toxicity tests carried out by Sowder (13) and Hawley (3), hot-water extracts from both heartwood and sapwood were found more toxic than corresponding cold-water extracts. This probably is due to the increased amount of extractives or to a difference in the nature of the extractives. The above workers (3) (13) also have found that in all cases the sapwood extracts are less toxic than corresponding heartwood extracts.

A very interesting example of the practical application and the importance of a thorough knowledge of water-soluble extractives of wood is the problem offered by "box scald." Box scald is the term applied to a certain type of injury to apples and pears resulting from contact with wooden containers (2). It is a brownish discoloration on the skin of the apple, with surface roughened as if it had been pressed against the board and subjected to abrasion. Tests have disclosed the fact that the injury is due to a water-soluble substance present in the heartwood but absent in the sapwood of Douglas fir. Western hemlock, western yellow pine, and Sitka spruce do not contain the injurious extractive.

The extractives in the tests reported in this paper were secured from air-seasoned and kiln-dried lumber. The kiln-dried stock was dried at a

kiln temperature of not higher than 180° F., which is not a severe dry-bulb temperature for Pondosa pine. It is reasonable to suppose that when kiln-drying lumber and subjecting it to temperatures that run as high as 200° F. (14) certain volatile constituents of the wood are driven off.

It is possible that the high temperatures used in kiln drying affect the durability of the wood. Koehler and Thelen (6) state: "Whether the removal of moisture in itself causes any chemical changes in wood substance is not known, but it is a well-established fact that heat, if severe enough, will cause a decided chemical change in wood with a corresponding reduction in strength." Little, however, is known of the relative durability of air-seasoned and kiln-dried stock.

Under ordinary conditions the decomposition of wood by heat does not progress to any extent until a temperature of 275° C. is reached (4). Up to this point all water is driven off, and possibly extraneous matter is volatilized, but no decided chemical change occurs in the wood. The products driven off at temperatures up to 280° C. to 290° C. are water, a small amount of acetic acid, and a trace of methanol. Active decomposition of wood into primary tar and primary charcoal occurs when the temperature is raised above this point. It is possible, however, that the same chemical changes may result in wood subjected to medium high temperatures for a long period as those changes that occur when the wood is subjected to a high temperature for a short period. The slow darkening of wood in contact with steam pipes (4) may be such a change.

The work of Bateman (1) on the effect of resin on durability leads us to believe that there is a definite relationship between the volatile oil content of resinous wood and durability. A certain relationship between resin, an extraneous component, and durability could be expected, since it is known that certain hydrocarbons, as toluene and benzene, etc., are very toxic to fungi (1). Experiments on the toxicity of terpenes and terpene alcohols by Bateman showed a strong retardation of the test organism, although no actual killing of the organism occurred. Cymene, the main constituent of spruce, turpentine, alpha pinene, the main constituent of gum turpentine from long-leaf pine, and beta pinene, the main constituent of gum turpentine from western yellow pine, showed a respective retardation of 98 per cent, 98 per cent, and 97 per cent.

Pine oil is extremely toxic. In tests (1) to determine the cause of the durability of an exceptionally durable, very resinous, heartwood long-leaf pine tie, it was found upon steam-distilling the tie that it contained ten times as much pine oil as was necessary to prevent the growth of decay organisms. Pine oil, one of the volatile oils found in yellow pine, is not present in "oleoresin." Oleoresin (4) is the viscous substance secreted

by the resin cells of the sapwood when the tree is wounded. Resin, which does contain small amounts of pine oil, is considered as an extraneous substance in the wood proper. Pine oil is probably an oxidation product, formed in the presence of air and water.

METHODS

Only actual service tests will give conclusive proof of the effects of kiln drying on lumber. Satisfactory service tests, however, cannot be carried out in less than 20 or 30 years. Laboratory tests on problems of this kind are merely indicatory of what the results probably will be. They are open to many criticisms, but at least they can be used as a basis and guide for conducting service tests.

Investigators in the past have used two principal methods in carrying on toxicity tests in the laboratory. In the first, the preservative is mixed with sawdust or injected into the wood and the fungus grown on a wood base, and, in the second, the preservative is mixed with some nutrient-agar solution. The nutrient solution varies from a malt to a beef or vegetable extract (9).

The growth of any particular fungus on different media varies and thereby renders noncomparable the results of toxicity tests carried out with the same fungus but on different media. The tendency (9) in late years has been to make all tests on a malt-agar medium, thus giving a uniformity in all tests. Therefore, all cultures in the following work were grown on a malt-agar medium. Hawley, Fleck, and Richards (3), in their investigations on toxic extracts of various woods, and Sowder (13), in his work on cedar extracts, used this type of medium.

Lenzites sepiaria (Fries), the fungus causing a brown cubical rot, was selected for the toxicity tests. Two factors were given special consideration in choosing a fungus for such tests: First, a fungus was wanted that actively attacks Pondosa-pine stock, in service; second, it was desirable to have a fungus that would be fairly sensitive to varying concentrations of preservative. Tests carried out by Schmitz (12) and Richards (10) indicate that *L. sepiaria* would give satisfactory results.

Characteristics of wood used in tests: Four pieces of western yellow or Pondosa pine were selected from which to secure the water-soluble extracts to be tested. These pieces were:

1. Kiln-dried heartwood.
2. Kiln-dried sapwood.
3. Air-seasoned heartwood.
4. Air-seasoned sapwood.

Care was used in selecting the stock to secure pieces of normal Pondosa pine, and all pieces were taken large enough to allow for any varying densities within the wood. Any pieces with pitch pockets, decay, knots, or blemishes of any kind were thrown out.

The number of rings per inch was then determined for each piece, as a large number of rings per inch sometimes indicates a dense wood. Within a given species the denser the wood, the more resistant it is to decay (15). To determine the moisture content and the specific gravity of each board used for each type of wood floor, an inch block was taken from the exact center of each. This position tends to give a truer average for the board.

Standard methods were used to compute the percentage of moisture and the specific gravity of each block (8).

Preparation of water-soluble extracts: The sawdust was ground into wood flour of such fineness that it passed through a 0.5 mm. sieve. Hot- and cold-water extracts were then prepared by weighing out duplicate sets of 300 gm. of each kind of wood flour; 3,000 cc. of distilled water at room temperature was then added to the one sample, and the same amount of boiling, distilled water was added to the duplicate. The cold-water sample was stirred intermittently and allowed to soak for 48 hours. At the end of this time, which was deemed long enough to leach out all water-soluble extracts from such fine particles of wood flour, the extract was drained off and the wood flour washed with 6,000 cc. of cold, distilled wash water, added to the soaking water. The hot-water sample was allowed to soak in a boiling-water bath for 3 hours, and then it was washed with 6,000 cc. of boiling, distilled water and the wash waters added to the soaking water.

The cold- and hot-water extracts were then passed through filter paper. The filtrate was evaporated down to 300 cc. at a temperature never exceeding 70° C. One cc. of filtrate, then, represented the amount of extract secured from 1 gram of wood flour. The extracts, with the exception of the kiln-dried and air-seasoned cold-treated heartwoods, filtered readily in from 2 to 12 hours. The two exceptions filtered slowly, requiring, respectively, 5 and 8 days.

No precipitation occurred in any of the filtrates before they were evaporated down. No distinct discoloration of any of the filtrates was noticeable. Each showed a slightly cloudy appearance, with a faint grayish to yellowish green tinge. As the filtrates were evaporated down to 300 cc., precipitates appeared. The amount and color of the precipitates varied with the type of filtrate.

DISCUSSION OF WATER-SOLUBLE EXTRACTS

The wood flour showed a slight darkening of the original color after having been treated with hot or cold water. Before treating, the kiln-dried heartwood showed a slightly darker light buff color than the air-seasoned heartwood. There was no difference in color between the kiln-dried and air-seasoned sapwood, either before or after treating.

In every instance the extracts from cold-treated wood flour were darker than the extracts from the corresponding hot-treated wood flour. Kiln-dried sapwood extracts were darker than the kiln-dried heartwood extracts and the same was true for the air-seasoned sapwood and heartwood extracts. The precipitates from the hot- and cold-treated kiln-dried heartwood and the hot-treated air-seasoned sapwood were the same color as the solution of the respective extracts. In all of the other extracts the color of the precipitate varied from that of the solution.

The amount of water-soluble material removed from the wood flour by the hot- and cold-water treatments was determined by measuring out 5 cc. of the extract in a watch glass. This sample was evaporated to dryness at a temperature of about 40° C. The residue was then weighed and the original volume (5 cc.) divided by the weight of the residue, since 1 cc. of the extract represents 1 gm. of wood flour, and this result, times 100, gave the percentage of water-soluble substance removed from the wood.

The amount of water-soluble material removed from each of the various extracts was as follows:

	Hot treated	*Cold treated*
Kiln-dried heartwood	0.1165 gm. or 2.33%	0.056 gm. or 1.12%
Kiln-dried sapwood	0.079 " " 1.58 "	0.045 " " 0.9 "
Air-seasoned heartwood	0.180 " " 3.6 "	0.089 " " 1.78 "
Air-seasoned sapwood	0.119 " " 2.38 "	0.063 " " 1.26 "

The hot-water treatment removed more water-soluble material from both the heartwood and sapwood than was removed by the cold-water treatment. Approximately twice as much material was dissolved by the hot water. The heartwood of the kiln-dried stock contained about a third more water-soluble material than the kiln-dried sapwood. The same was true of the air-seasoned heartwood and sapwood. This was to be expected since the densities of the heartwood were considerably greater than those of the sapwoods.

Litmus tests of the different extracts gave a slightly acid reaction for the hot- and cold-treated air-seasoned sapwood. The other extracts gave no reactions, whatever.

TOXICITY TESTS

Methods: The toxic qualities of each of the extracts were tested by the Petri-dish method. (2). A malt-agar medium was used. Concentrations of 10, 25, 50, 75, and 100 per cent of each of the extracts were prepared for the tests. Each of these concentrations was prepared in series of 5, so that the results would represent the averages of a large number of Petri-dish tests. Therefore, the results in this paper are based on a total of 240 Petri-dish cultures.

For the 10 per cent and 25 per cent concentrations a 1.5 per cent malt-agar medium was used. The malt agar consisted of 1,000 cc. of distilled water, 25 gm. of Trömmer's malt extract, and 15 gm. of bacto-agar. The medium used for the 50 and 75 per cent concentrations was a 3 per cent malt-agar solution consisting of 1,000 cc. of distilled water, 25 gm. of Trömmer's malt extract, and 30 gm. of bacto-agar. A 3 per cent malt-agar medium was used for the 50 and 75 per cent concentrations instead of the 1.5 per cent medium, so that the concentration could be gelatinized.

The 10 per cent concentrations consisted of 2 cc. of wood-flour extract, 1 cc. of distilled water, and 17 cc. of 1.5 per cent malt agar. The 25 per cent concentrations consisted of 5 cc. of wood-flour extract and 15 cc. of malt agar; the 50 per cent concentrations of 10 cc. of wood-flour extract and 10 cc. of 3 per cent malt agar; and the 75 per cent concentrations of 15 cc. of wood-flour extracts and 5 cc. of 3 per cent malt agar. The 100 per cent concentrations consisted of 20 cc. of wood-flour extract.

The various concentrations were measured out in test tubes, plugged with a cotton plug, and sterilized at 100° C. for 30 minutes on 3 successive days. Each tube was then shaken thoroughly, so as to secure a thorough and even mixture, and then poured into a Petri dish. Great care was taken

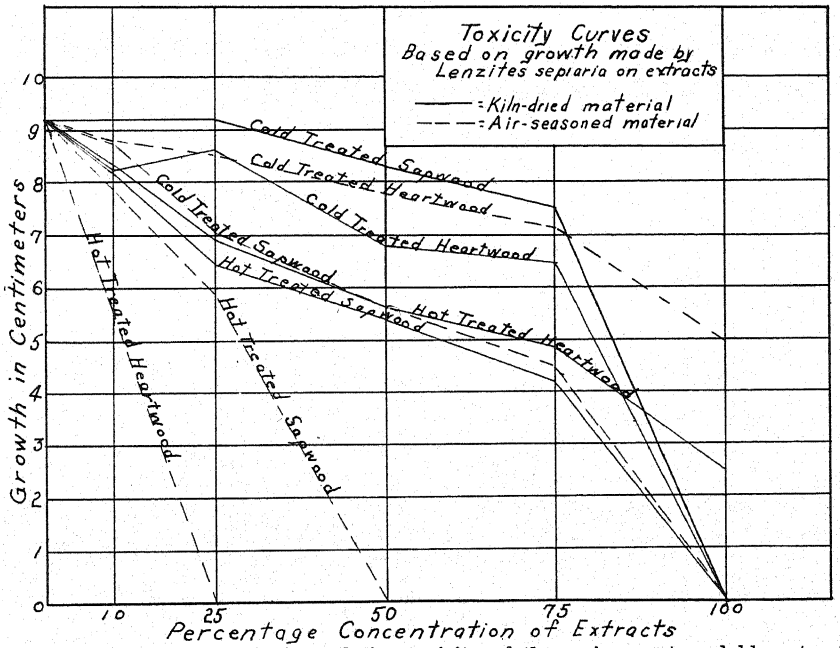


FIG. 1. Toxicity curves showing relative toxicity of the various water-soluble extracts of Pondosa pine based on the growth made by *Lenzites sepiaria* on different concentrations of the extracts.

to keep all dishes free from contamination. All contaminated dishes were discarded.

Each dish was then inoculated with a 0.5 cm. square of a fresh vigorously growing culture of *Lenzites sepiaria*. The culture of the fungus was secured from a transfer in possession of Dr. E. E. Hubert, Forest Products Laboratory, Moscow, Idaho. The culture was originally obtained from a hemlock log at the Forest Products Laboratory, Madison, Wisconsin. The inoculum was grown on a 1.5 per cent malt-agar medium.

To determine the exact effect of the extracts on the growth of the fungus, a series of 5 controls was made for each type of extract. The medium of the control dishes consisted of 20 cc. of 1.5 per cent. malt agar.

The cultures were then placed in a culture case and allowed to grow until the fungus reached the edges of the Petri dish. The culture case was kept at a temperature of about 22° C. Measurements of the diameter growth were taken at various intervals. To secure a correct growth reading of each dish, two measurements were taken at right angles to each other and averaged. The controls required approximately 22 days to reach the edges of the Petri dishes.

GROWTH OF CULTURES

Some of the cultures showed no growth, whatever. After 10 days, to determine whether the fungus had been killed or growth merely inhibited, a transfer was made from those cultures showing no growth and placed on a test-tube slant of 1.5 per cent malt agar. All of the cultures that failed to grow, and are marked with ([a]), in table 1, recovered within 3 weeks when transferred to slants; growth was merely inhibited. Those cultures marked with ([b]) failed to recover; in these cases the fungus was killed by the toxic properties of the extracts. The 100 per cent concentrations of hot-treated air-seasoned sapwood and hot-treated air-seasoned heartwood were the only two extracts that actually killed the fungus.

Photographs were taken of representative cultures of the concentrations of the different types of water-soluble extracts. The plates of each concentration selected for photographing showed a medium condition of growth and were typical for that concentration. Figure 2 shows these cultures, together with the control plate of 1.5 per cent malt agar. They give a comprehensive comparison of the growth of the fungus and the characteristics of the growth, especially as to its luxuriance, diameter growth, subgrowth, and evenness.

The growth of *Lenzites sepiaria* on pure malt agar tends to form a raised ring of aerial growth of an inch radius about the inoculum. The aerial mycelium is white to gray and tinged with yellow, as it ages. The

culture tends to show a bunchy rather than a uniformly even growth of mycelium.

The percentage of retardation of the growth made by the culture was secured by dividing the growth of the culture by that of the growth on the control and subtracting the result from 1 and multiplying by 100.

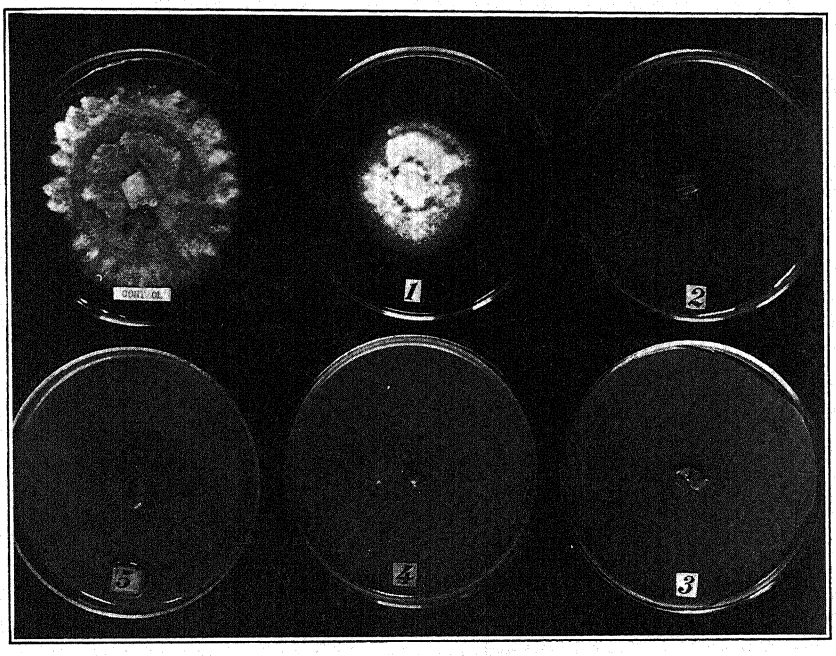


FIG. 2. Photograph showing growth of *Lenzites sepiaria* made on control of 1.5 per cent malt agar and on various concentrations of hot-water-soluble extracts obtained from the heartwood of air-seasoned western yellow pine. Nos. 1, 2, 3, 4, and 5 represent the growth on concentrations of the extracts of 10, 25, 50, 75, and 100 per cent, respectively.

The growth of the fungus on the concentrations of the various water-soluble extracts and also the percentage of retardation are tabulated in table 1. The curves in figure 1 are based on the growth data in table 1. The graph shows the relative toxicity of each type of extract as removed from the wood of Pondosa pine.

Of all the extracts, the hot-treated air-seasoned heartwood extract was the most toxic to *Lenzites sepiaria*. The 10 per cent concentration showed a retardation of 41 per cent, and the 25, 50, and 75 per cent concentrations showed a total inhibition of the growth of the fungus. The cold-treated kiln-dried sapwood showed the least toxicity to the fungus.

TABLE 1.—*Growth and percentage retardation of Lenzites sepiaria on the various concentrations of the water-soluble extracts of Pondosa pine after 22 days*

Water treatment	Type of wood flour from which water-soluble extract was secured	Control	Average growth in Petri dishes expressed in centimeters and percentage retardation of extracts									
			10% Conc.		25% Conc.		50% Conc.		75% Conc.		100% Conc.	
			Gr.	Ret.	gm.	Ret.	gm.	Ret.	gm.	Ret.	gm.	Ret.
Hot	Kiln-dried heartwood	9.2	8.31	10	6.92	25	5.62	39	4.83	47	2.45	73
Cold	"	9.2	8.2	11	8.6	7	6.78	26	6.46	30	0	100[a]
Hot	Kiln-dried sapwood	9.2	8.16	11	6.42	30	5.38	42	4.2	54	0	100[a]
Cold	"	9.2	9.2	0	9.2	0	8.28	10	7.48	19	0	100[a]
Hot	Air-seasoned heartwood	9.2	5.42	41	0	100[a]	0	100[a]	0	100[a]	0	100[b]
Cold	"	9.2	8.8	4	8.52	8	7.75	16	7.12	23	4.92	47
Hot	Air-seasoned sapwood	9.2	7.86	15	5.86	36	0	100[a]	0	100[a]	0	100[b]
Cold	"	9.2	8.74	5	6.98	24	5.62	39	4.48	51	0	100[a]

[a] Fungus transferred to malt-agar slant recovered.

[b] Fungus transferred to malt-agar slant failed to recover.

The plotted growth data bring out clearly that in every case the hot-water extracts of western yellow pine are more toxic to *Lenzites sepiaria* than the corresponding cold-water extracts.

No very marked difference was noticeable between the toxicity of the heartwood extracts and the corresponding sapwood extracts. This was to be expected, since there is not a great deal of difference in the durability of western yellow pine heartwood and sapwood. The hot-treated air-seasoned-heartwood and the cold-treated kiln-dried-heartwood extracts are more toxic than the corresponding sapwood extracts. The hot-treated kiln-dried-heartwood extract is slightly less toxic than the hot-treated kiln-dried-sapwood extract. This may be accounted for by the fact that, due to trouble in controlling temperatures, the cultures of the hot-treated heartwood extract were grown at a temperature of about 27° C., which is perhaps the optimum temperature for the growth of *Lenzites sepiaria*. After temperature troubles had been adjusted all cultures were grown at 22° C.

The cold-treated air-seasoned-heartwood extract shows a lower toxicity than the cold-treated air-seasoned-sapwood extract. This seems to be at variance with the work done by Hawley, Fleck, and Richards (3) and Sowder (13), in which the heartwood extracts were found to be more toxic in every case than the corresponding sapwood extracts. This reversal can be accounted for only on the assumption that, since the heartwood and sapwood probably did not come from the same tree and since there seems to be so little difference between the toxicity of the heartwood and sapwood water-soluble extracts, the variance may be within the limits of individual characteristics of the wood from different trees.

The work of Hawley, Fleck, and Richards (3) shows that in species where there is little difference between the durability of the heartwood and the sapwood there is also little difference between the toxicity of corresponding heartwood and sapwood extracts.

After plotting the growth data it was noticed that the water-soluble extracts obtained from air-seasoned wood showed a slightly greater toxicity to *Lenzites sepiaria* than the corresponding kiln-dried extracts. This would seem to indicate that the temperatures used in kiln drying western yellow pine may have a slight effect on the chemical properties of the wood. It may be that certain elements in the wood are volatilized.

STUDY OF THE WATER-TREATED WOOD FLOUR

Methods: To determine to what extent *Lenzites sepiaria* can grow on the water-treated wood flour of Pondosa pine, a series of toxicity tests was carried out. The wood flour was first dried to a constant weight at a temperature of 100° C. This required about 48 hours. It was then weighed out in samples of approximately 4 gm. each and in series of 5. The sam-

ples were placed in 150 cc. wide-mouth, flat-bottom extraction flasks of known weights. To find the exact effect of the fungus on the treated wood flour a series of controls of untreated wood flour was made and handled exactly the same as the treated wood-flour samples.

Twenty-five cc. of sterile distilled water were added to each flask. The flasks were next inoculated with a vigorously growing culture of *Lenzites sepiaria* and each flask was then plugged with a cotton plug. To keep the loss of moisture through the plug at a minimum the mouth of each plugged flask was covered with waxed paper and tied securely. The flasks were then placed in a culture chamber and incubated for a period of 5 months at approximately 22° C. After 3 months it was noticed that the cultures were becoming dry, so 5 cc. of sterilized distilled water were added to each flask. Table 2 gives the approximate amount of mycelial growth visible on each culture and the average percentage loss of weight for each type of wood flour.

The percentage loss of weight was computed by dividing the loss in weight of the oven-dry wood flour at the end of the incubation period by the original oven-dry weight of the wood flour.

Discussion of Results: The data of table 2 show that the sapwood of Pondosa pine has less resistance to *Lenzites sepiaria* than the heartwood. The mycelial growth was slightly better on the sapwood cultures than on the heartwood cultures.

The percentage loss of weight based on the original oven-dry weight of the wood flour should be greater for hot-water-treated samples than for the corresponding cold-water-treated samples, because more of the toxic water-soluble material was removed by the hot-water treatment. The loss of weight of the hot-treated kiln-dried heartwood is slightly more than the corresponding cold-water-treated kiln-dried heartwood. The same is true for the hot- and cold-air-seasoned heartwood. Both the hot- and cold-treated air-seasoned sapwood samples and the control show a larger loss of weight than any of the other types of wood flour. The water-soluble extracts of the air-seasoned sapwood gave an acid reaction and, since the growth of fungi is favored by a slightly acid medium, this may account for the good growth of the fungus on the air-seasoned sapwood.

The kiln-dried sapwood cultures show an actual retardation of *Lenzites sepiaria* by the water treatment and do not correlate with the results of the toxicity tests of the water-soluble extracts. The growth of the cultures was irregular. Of the 10 cultures, 3 showed no mycelial growth, 4 showed a little growth, 2 showed medium, and 1 flask had good growth. No reasons can be given that would account for the results obtained in this set of cultures.

TABLE 2.—*Growth of Lenzites sepiaria on the treated wood flour of Pondosa pine*

Water treatment	Wood flour treated	Visible mycelial growth	Percentage loss of weight based on original oven-dry weight	Basis no. of tests
Hot	Kiln-dried heartwood	Medium	12.6	5
Cold	"	Slight	10.5	5
Control	"	"	11.8	5
Hot	Kiln-dried sapwood	"	4.6	5
Cold	"	Medium	10.6	5
Control	"	Good	24.0	5
Hot	Air-seasoned heartwood	Medium	13.1	5
Cold	"	Slight	8.6	5
Control	"	"	14.6	5
Hot	Air-seasoned sapwood	Medium	17.4	5
Cold	"	"	19.9	5
Control	"	"	16.6	5
Total				60

The toxicity of the water-soluble extracts of Pondosa pine is low, and, to secure reliable results from toxicity experiments with hot-and cold-water-treated wood flour, it will be necessary to run a much larger series of tests than it has been possible to run in the preparation of the data for this paper. It would also be advisable to incubate the wood-flour cultures for a longer period than time allowed in this study.

SUMMARY

1. The hot-water extracts of Pondosa pine are more toxic to *Lenzites sepiaria* than corresponding cold-water extracts.

2. In general, the water-soluble-heartwood extracts seem to be slightly more toxic than the corresponding sapwood extracts, but no pronounced difference in the relative toxicity of the two types of extracts was noticed.

3. The water-soluble extracts obtained from air-seasoned material were more toxic to the fungus used than corresponding water-soluble extracts obtained from kiln-dried material. This would indicate a loss of certain volatile materials toxic to *L. sepiaria* brought about by the temperatures used in kiln drying Pondosa pine.

618 REALTY BUILDING,
SPOKANE, WASH.

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HETEROTHALLISM IN PHYTOPHTHORA[1]

LEON H. LEONIAN

Ashby (1) was the first to observe that when a strain of *Phytophthora faberi* Maubl. was grown with a strain of *P. palmivora* Butler or of *P. parasitica* Dastur, oospores developed, while no such bodies formed when it was grown alone. He suggested the possibility of heterothallism in *P. faberi*. Gadd (8) substantiated Ashby's findings and strongly subscribed to the theory of heterothallism. However, Lester-Smith (10) discarded the possibility of heterothallism and suggested that oospore production in paired cultures was due to a biochemical stimulation of the one strain by the other. Ashby (3) in a later work inclines towards the hypothesis advanced by Lester-Smith and subordinates heterothallism to autogamy; he considers chemical changes caused by the association of two different strains as being the chief underlying factor in the production of the sexual bodies. Narasimhan (11) has recently demonstrated that of the 7 Phytophthora cultures (presumably *P. palmivora*) collected by him and tested under laboratory conditions, 4 belonged to one sex and 3 to the other. When grown by themselves or when paired with strains of the same sex, no oospores resulted. Similarly, when *P. parasitica* or *P. meadii* McRae were grown with *P. arecae* (Colem.) Peth., oospores formed, but no such bodies could be found when these cultures were grown by themselves.

All clear-cut cases of heterothallism in Phytophthora have been reported in the *Phytophthora omnivora* group. Clinton's (7) claim that he obtained hybrid oospores by growing *P. infestans* de Bary and *P. phaseoli* Thaxt. together is not conclusive, especially in view of the fact, as shown later in this paper, that the size of oogonia and oospores in the first-generation crosses is controlled by the female strain and that the antheridial strain has little or no influence on the size of these bodies. Furthermore, *P. phaseoli* is homothallic and produces great numbers of oospores in pure cultures; *P. infestans* is also homothallic, at least in so far as our present knowledge goes, but forms its sexual organs only sporadically and quite sparsely. It is, therefore, very difficult, if not impossible, conclusively to demonstrate that hybridization can be brought about between these two species, unless one is able to germinate the oospores and to follow the morphology and the behavior of the second-generation hybrids. The same thing applies to alleged crosses between *P. infestans* and *P. cactorum* (Con. & Leb.) Schroet. If one or the other of any paired culture happens to be homothallic, all claims about hybridization or possible mutual stimulation lose their signifi-

[1] Published with the approval of the Director, West Virginia Agricultural Experiment Station.

cance. Ashby's (2, 3) statement that he observed large oospores when *P. cinnamomi* was grown with *P. parasitica* and (4) with *P. cryptogea* Peth. & Laff. or *P. richardiae* Buism. should not be accepted as sound evidence in support of either heterothallism or the biochemical stimulation theory. All three of these species are homothallic; Ashby (5), himself, has observed oospores in pure cultures of *P. cinnamomi* Rands; all strains of this species, so far examined by the writer, seem to be homothallic. Relative abundance of such bodies is not at all significant, especially when one is advocating the hypothesis that heterothallism is not the controlling factor in oospore production and that the solution of the phenomenon is to be sought in biochemical stimulations.

The present paper is an effort to extend a little farther our knowledge on heterothallism in Phytophthora. Much remains to be clarified and more fundamental research on a larger scale is essential before indisputable conclusions can be proposed.

It was realized at the beginning that no conclusive data could be presented without employing a large number of strains in the experimental work. Accordingly, some 85 cultures of the *Phytophthora omnivira* group, occurring largely in the Tropics, were collected and paired in hundreds of cultures. It soon became evident that variability was as great a factor in the sexual relationships of these fungi as it is in morphological characters and in physiological reactions. Eventually four distinct groups were segregated: 1, homothallic; 2, heterothallic; 3, inconstant; and 4, neutral. The forms of the inconstant group were at times heterothallic and at times neutral; when paired with the proper sex they sometimes formed oospores and sometimes they did not. The neutral forms produced no oospores, regardless of whether they were grown with male or female strains. After the organisms belonging to the first, third, and fourth groups were eliminated, 48 cultures still remained. One-half of these consisted of males and the other half of females. Any of the male strains when mated with any of the female strains gave rise to oogonia, whereas males paired with males and females with females, or else males or females grown by themselves, failed to produce any oogonia.

Oatmeal agar in Petri dishes was used throughout this work. Many other agars were tried and discarded as they failed to induce sexual bodies. It makes no difference how the two pieces of inoculum are planted in the dish; they may be planted together in the same spot or they may be planted any distance apart in the dish; oogonia will form whenever the two hyphae of opposite sexes touch or mix.

The heterothallic strains of Phytophthora here studied are classified in the following table according to their sexuality and origin. In case of some strains there are no data available as to their exact origin and hosts;

however, they are all tropical forms, and there is no doubt concerning their taxonomic status. While all organisms listed in this table belong to *Phy-*

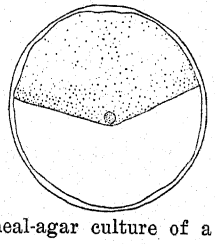


FIG. 1. Sketch of an oatmeal-agar culture of a second-generation transfer, *Phytophthora palmivora* 1 × *P. palmivora* 2. Shaded region represents the oogonia-bearing sector, while the unshaded region consists of a pure culture of *P. palmivora* 1 bearing no oogonia.

TABLE 1.—*Male and female strains of Phytophthora omnivora*[a]

Female strains	Male strains
P. faberi 4, cocoanut	*P. faberi* 1, cocoanut
P. " 5, papaya fruit	*P.* " 2, "
P. " 6, cocoanut	*P.* " 3, "
P. " 7	*P.* " 8
P. " 10	*P.* " 9
P. " 14	*P.* " 11
P. palmivora 1, cocoanut	*P.* " 12
P. " 4, Borassus	*P.* " 13
P. " 9	*P.* " 15
P. parasitica 1, tomato, Morgantown, W. Va.	*P.* " 16
P. " 2	*P. palmivora* 2, cocoanut, Porto Rico
P. " 4, Citrus, Philippines	*P.* " 3, Sabal, Porto Rico
P. " 5, Hibiscus, Java	*P.* " 5, grapefruit, Porto Rico
P. " 7, Bryophyllum, Porto Rico	*P.* " 6, Hevea
P. " 8	*P.* " 7, Ashby's strain
P. " 10	*P.* " 8
P. " 11, Bryophyllum, Bermuda	*P. parasitica* 3, eggplant, Philippines
P. " 13	*P.* " 6, tomato, Porto Rico
P. " 14	*P.* " 9, Vigna, Java
P. " 15—II, III, IV, V, VI, VII	*P.* " 12
P. " 16	*P.* " 15—I
P. " 17	*P.* " 18
P. terrestris, Sherbakoff's strain	*P.* " 19
P. manoana, Sideris's strain	*P. nicotianae*, Holland strain

[a] Most of the organisms listed in this table were secured from C. M. Tucker, Carl Hartley, American Type Culture Collection, and Centraalbureau voor Schimmelcultures, Baarn, Holland.

tophthora omnivora group, their older names are retained here for the sake of convenience in making comparisons.

One interesting thing brought out in the foregoing table is that of the 7 dissociants of *Phytophthora parasitica* 15, 6 are female and 1 is male. As these 7 strains were separated long before their sexual or heterothallic tendencies were tested, the writer has no direct evidence to show the exact sex of the original strain; however, since only 1 strain of the 7 is male, it is safer to assume that the sexual tendency of the original culture was toward femaleness. All 6 of the female strains are sparse oogonia formers, which seems to indicate that their femaleness is not very pronounced; but the 1 male strain has a strong male tendency and when mated with such a prolific female as *P. palmivora* 1, it is able to supply all the antheridia that this female strain needs for its numerous oogonia. It may be stated that the original culture might have been a mixture of two organisms and that *P. parasitica* 15—I was an entirely different culture from the other 6; this is possible but not probable. It still remains to be demonstrated that 2 distinct strains of any given species can be carried together in pure culture

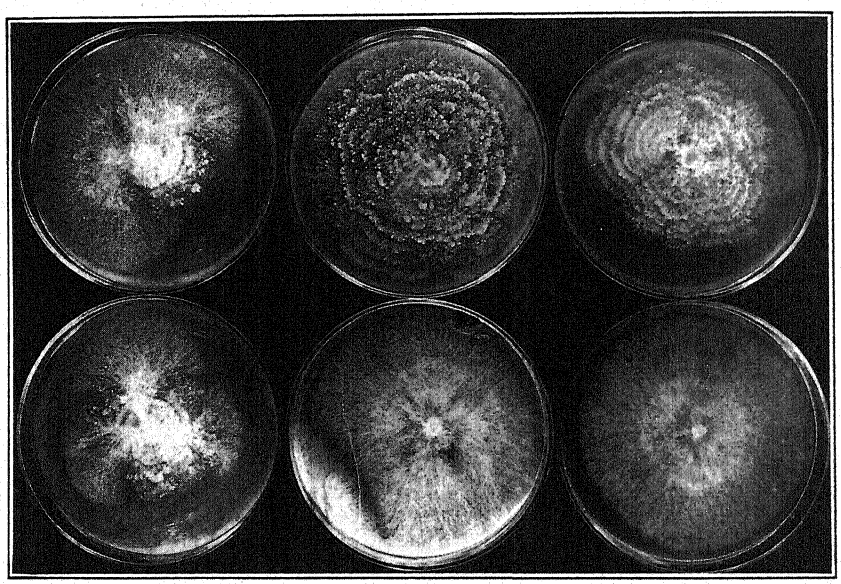


FIG. 2. Upper right, *Phytophthora parasitica* 15; lower right, *P. palmivora* 4; pure cultures showing the growth habit of these two fungi. Upper middle, mixed culture; *P. parasitica* 15 has outgrown *P. palmivora* 4, the latter appearing as a faint growth in the center of the colony; lower middle, *P. palmivora* 4 has outgrown *P. parasitica* 15. Left row, *P. parasitica* 15 and *P. palmivora* 4 splitting apart. In case of the middle two and the left two cultures, transfers were made directly from the oogonia-bearing regions in oatmeal agar, to plates of malt-extract agar.

for any length of time. Usually after 1 or 2 transfers one or the other strain will be left behind. (Figs. 2 and 3.) (Also note similar phenomena observed by the writer (4) in case of *Fusarium moniliforme*.) *Phytophthora parasitica* 15 has been in pure culture for many years and has been transferred from plate to plate and from tube to tube so many times that the likelihood of a simultaneous transfer and subsequent harmonious growth of two different strains of fungi is extremely remote. Furthermore, all seven strains of this fungus show almost identical reactions and many points of close similarity and constitute, in all probability, the different dissociants of the same fungus. Since almost every morphological and physiological characteristic of a given fungus may be materially altered by the dissociation phenomena, there is no reason to suppose that sexuality constitutes an exception.

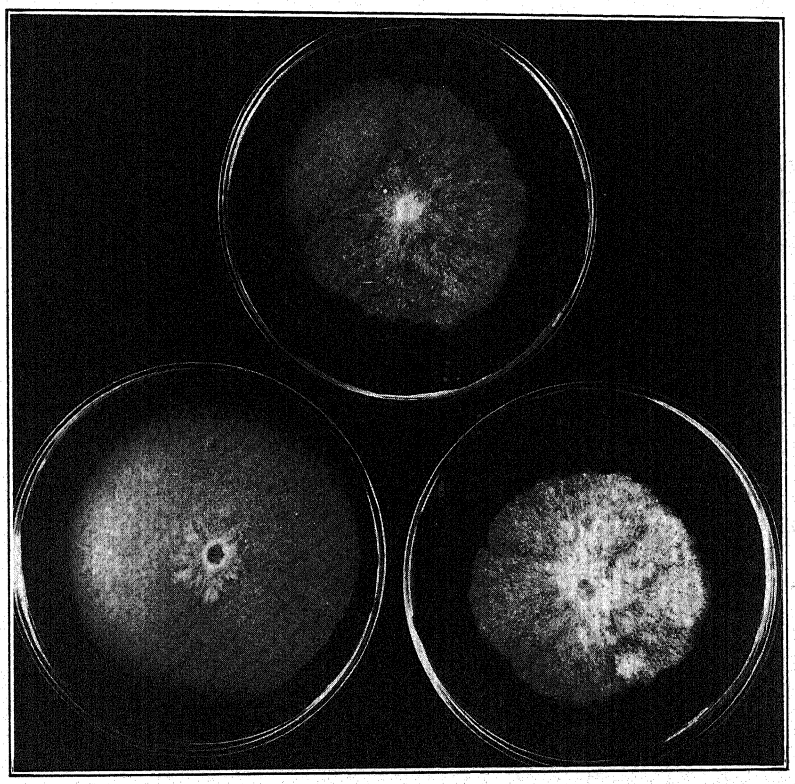


FIG. 3. Second-generation transfers from oogonia-bearing regions on oatmeal agar to malt-extract agar, *Phytophthora faberi* 1 × *P. parasitica* 5. Upper picture, *P. faberi* 1 sectoring away from *P. parasitica* 5. Lower left, *P. faberi* 1 has outgrown *P. parasitica* 5; the latter can be seen as a small growth confined to the center of the colony. Lower right, *P. parasitica* 5 has completely outgrown *P. faberi* 1.

The oogonial and the antheridial strains were identified by the following method: oatmeal-agar plates were poured and sterilized. After the agar hardened, a strip about 1 cm. wide was cut away through the center of the plate, thus leaving a channel that separated the two halves of the oatmeal agar. These two halves were then inoculated by transferring one strain to the one half and the opposite strain to the other half. A sterilized transparent agar consisting of 5 gm. of dry malt extract, ½ gm. each of magnesium sulphate and dihydrogen potassium phosphate, and 20 gm. of agar agar in 1,000 cc. of distilled water was poured into this channel and allowed to harden there. As the hyphae from the two halves of the oatmeal agar grew into this transparent agar, met there and formed their oogonia, the plates were inverted under the microscope, and the oogonial branch was located and then traced to its source. The malt-extract agar alone is unable to induce oogonia; in fact, the opposite sexes often repel each other if grown on this agar. But, when the hyphae were allowed to feed on oatmeal, the carrying-over effect of the substance essential for oogonial production was sufficient to induce the formation of such bodies on the transparent agar. This carrying-over effect has a radius of about 1 cm., beyond which no oogonia form. This was demonstrated by the following method: malt-extract agar was poured in Petri dishes and sterilized; after it hardened,

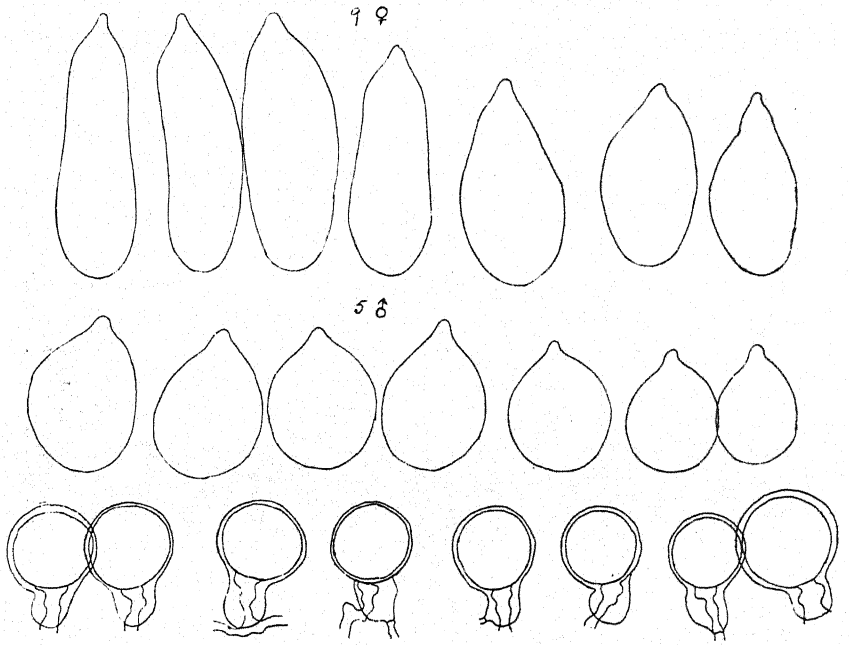


FIG. 4. Upper row, sporangia of *Phytophthora palmivora* 4; middle row, sporangia of *P. parasitica* 15; lower row, oogonia. Drawn to the scale.

large discs were cut away from the center of the plates by means of a cork borer with an opening of $2\frac{1}{4}$ cm. The vacant circular area thus formed was filled with sterilized oatmeal agar and inoculated with the male and female strains. Oogonia formed not only in the oatmeal agar but also in the surrounding malt-extract agar for a radius of 1 cm. around the oatmeal.

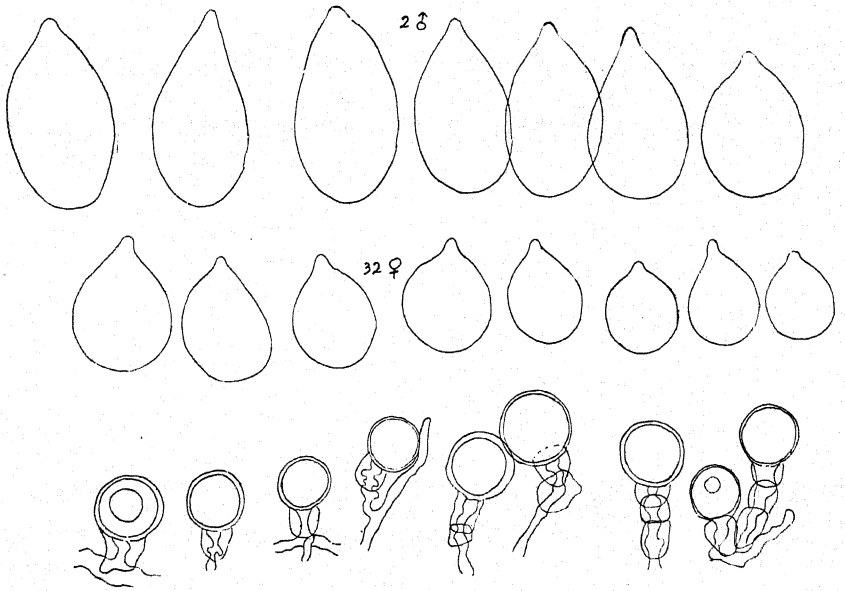


FIG. 5. Upper row, sporangia of *Phytophthora palmivora* 2; middle row, sporangia of *P. parasitica* 14; lower row, oogonia. Note that some of the oogonia possess two and three antheridia in a row.

Aside from the evidence of direct microscopic examination, two indirect methods were used to substantiate the microscopic determination. The size of oogonia is controlled by the female strain; a given female strain, which produces only small oogonia, will continue to do so no matter with what male strain it is mated. For instance, *Phytophthora palmivora* 4, when mated with any of the male strains, forms oogonia that measure 35 μ; whereas, *P. parasitica* 14, mated with the same male strains, will form oogonia that will average not more than 28 μ (Fig. 6). This is indirect but substantial evidence that *P. palmivora* 4 or *P. parasitica* 14 are the oogonia-forming strains and that any strain that can react with these must be male. The relative quantity of oogonial production is another safe indicator of sex. For instance, *P. palmivora* 1, when mated with any of the opposite strains, forms countless oogonia; whereas, *P. parasitica* 2, mated with the same male strains, invariably gives rise to few oogonia. The logical assumption, therefore, is that *P. palmivora* 1 and *P. parasitica* 2

are oogonial strains and all cultures that will react to them to form oospores must be antheridial strains.

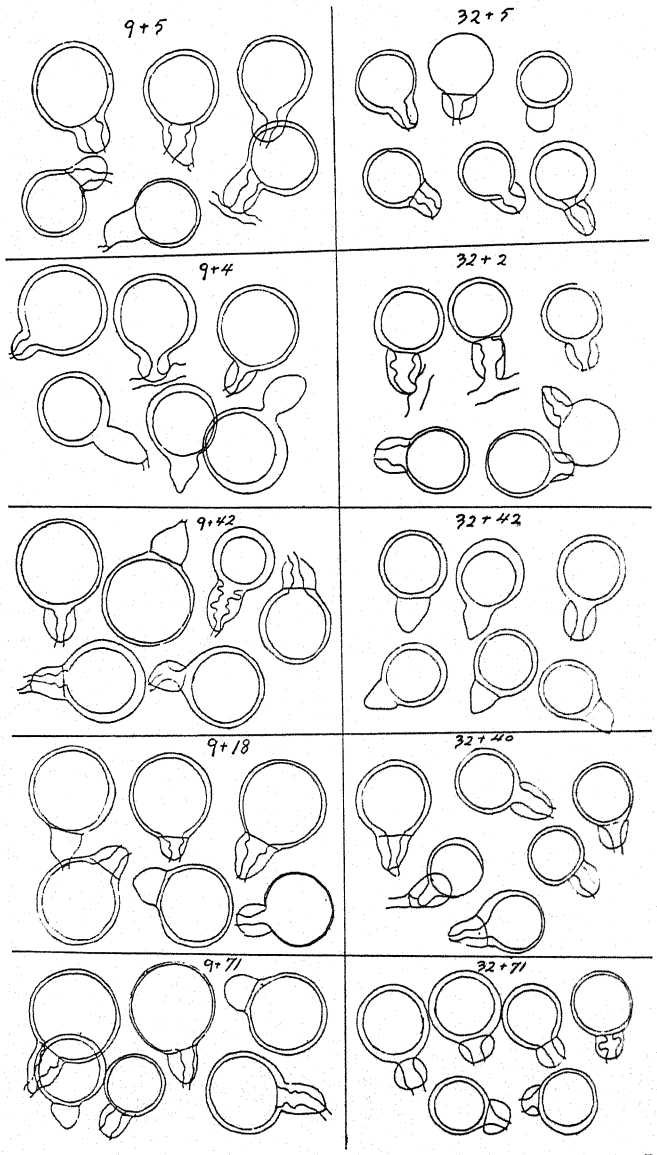


FIG. 6. Oogonia of *Phytophthora palmivora* 4 and of *P. parasitica* 14. Left column, *P. palmivora* 4 mated with *P. faberi* 2 (9×5), *P. faberi* 1 (9×4), *P. faberi* 13 (9×42), *P. palmivora* 6 (9×18), and *P. parasitica* 18 (9×71). Right column, *P. parasitica* 14 mated with *P. faberi* 2 (32×5), *P. palmivora* 2 (32×2), *P. faberi* 13 (32×42), *P. faberi* 11 (32×40), and *P. parasitica* 18 (32×71). Note the uniformly larger-size oogonia of *P. palmivora* 4 and the uniformly smaller-size oogonia of *P. parasitica* 14.

The first sexual bodies to form in paired cultures are to be found along the line where the two opposite hyphae first come together. The oospores, however, do not, by any means, confine themselves to this narrow region but gradually spread out and eventually may cover the entire plate. This indicates that the mycelium of one strain can grow readily into a region already occupied by the hyphae of the opposite strain.

Age of the mycelium is not a limiting factor in oogonial formation; fusion will occur if either young or old hyphae of the opposite sexes are brought together. This was demonstrated by the following method: oatmeal-agar plates were poured and sterilized; some of these were inoculated with the male strain alone, others with the female strain. One week later the entire culture, either male or female, consisting of mycelium and agar, was removed from one plate and inverted over the culture of the opposite sex in another plate; 2 and 3 weeks later the same thing was repeated with the remaining cultures. When examined, oogonia were found in all of the cultures thus treated.

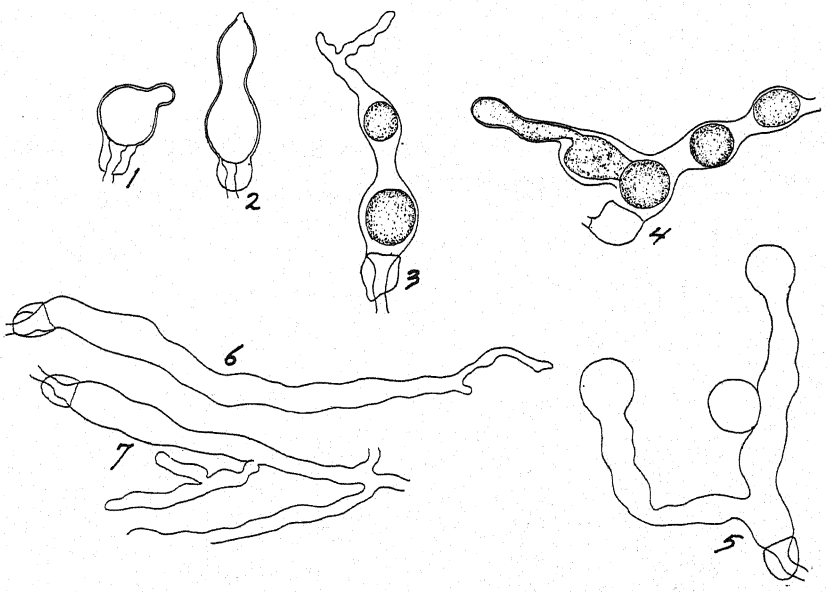


FIG. 7. Germination of oospores of *Phytophthora palmivora* 4. The round bodies in 3 and 4 probably are secondary oospores. The germination occurs *in situ;* only a small percentage of the oospores, however, shows any tendency towards germination.

Although, oogonia formed invariably whenever the two opposite sexes were mated together, transfers made from such cultures did not always give rise to oogonia. This was tested with the most prolific cultures for five generations. In the first-generation transfers the percentage of cultures

having formed no oogonia was quite small. For the second generation transfers selections were made from cultures which showed the greatest abundance of sexual bodies. This was continued until the fifth generation. It was noted that with each successive transfer the percentage of cultures showing no oogonia increased until, after the fourth-generation transfer, no oospores formed, even though a microscopic examination of the inoculum in each case showed an abundance of plump, normal-looking oospores. Some of the cultures manifested a sharp sectoring (Fig. 1), one part of the colony being full of oospores and the other part showing none. Transfers made from this latter region gave rise to a pure colony of the female strain which, when subsequently mated with the same male strain, gave rise to an abundance of oogonia. This shows that constant association of the two sexes tends to bring about a temporary sexual incompatibility, so that, despite the intimate presence of the two sexes, no fusion takes place; there may even be an actual repulsion. Such a behavior may seem strange if we were to regard sexuality as a constantly vital and fundamental phenomenon in these organisms; but if we regard chemotropism as the basis of all heterothallic and parasitic affinities, the foregoing behavior of the sexes will not seem quite so mystifying. Burgeff (6) has already advanced the hypothesis that certain species of Mucorales have become parasitic upon other species as a result of attempts to bring about hybridization. Assuming, therefore, that heterothallism can be explained on the grounds of chemotropic affinities or parasitic tendencies, it will not be difficult to understand that sometime in the relationship of the two sexes periodic changes in the protoplasm of one or the other sex will bring about resistance against the advances of the opposite sex, so that sexual affinities are submerged and a temporary sexual neutrality is brought about. The constant asociation of the opposite sex will serve to uphold this neutrality; but when the two sexes are separated the former affinities once more come to the fore. This explanation may sound fanciful, but it has an analogous case in the relationship of bacteria and bacteriophage where a constant association brings about a temporary immunity in bacteria towards the bacteriophage, but when this association is terminated the immunity disappears.

THE EFFECT OF DYES ON THE GROWTH REACTION OF DIFFERENT SEXES

In order to find out if there is any correlation between sexuality and the growth reaction of the different strains of Phytophthora used in this work, two dyes, crystal violet and malachite green, were used in minute quantities. A nutrient solution was prepared consisting of the following ingredients: 2 gm. of protease peptone, 0.5 gm. each of dihydrogen potassium phosphate and magnesium sulphate, 0.2 gm. succinic acid, 5 gm. of

dextrose, and 1,000 cc. of distilled water. This solution was separated into two lots; to one was added enough crystal violet to give a proportion of 1 part of the dye in 1,000,000 parts of the nutrient solution. To the second lot was added enough malachite green to give a proportion of 1 part of the dye in 2,000,000 parts of the nutrient solution. These were tubed, 5 cc. of the solution in each tube, and sterilized. Transfers were made to these tubes from vigorously growing colonies in Petri dishes by cutting inoculum discs from the outermost edge of the colony by means of a cork borer of 4-mm. bore. Readings were made 2 weeks later. The results are given in tables 2, 3, and 4.

The foregoing 23 organisms were the only ones unable to grow in the presence of 1 part of crystal violet in 1,000,000 parts of the nutrient solution.

TABLE 2.—*Organisms unable to grow in the presence of 1: 1,000,000 crystal violet*

Female strains	Male strains
P. faberi 4	*P. faberi* 1
P. " 5	*P.* " 3
P. " 6	*P.* " 8
P. " 7	*P.* " 11
P. " 10	*P.* " 12
P. " 14	*P.* " 13
P. palmivora 1	*P.* " 15
P. " 4	*P.* " 16
P. parasitica 15—III	*P. palmivora* 5
P. " 16	*P.* " 6
P. manoana	*P.* " 7
	P. " 8

TABLE 3.—*Organisms unable to grow in the presence of 1: 2,000,000 malachite green*

Female strains	Male strains
P. faberi 4	*P. faberi* 3
P. " 5	*P.* " 8
P. " 6	*P.* " 9
P. " 7	*P.* " 12
P. " 10	*P.* " 13
P. palmivora 1	*P.* " 15
P. " 4	*P.* " 16
P. " 9	*P. palmivora* 2
P. parasitica 5	*P.* " 6
P. " 16	*P.* " 7
	P. " 8
	P. parasitica 15—II
	P. " 18

TABLE 4.—*Organisms able to grow in the presence of 1: 2,000,000 malachite green*

Female strains	Male strains
P. faberi 14	*P. faberi* 1
P. parasitica 2	*P. palmivora* 3
P. " 4	*P. nicotianae*
P. " 7	*P. parasitica* 6
P. " 8	*P.* " 9
P. " 10	*P.* " 12
P. " 13	*P.* " 19
P. " 14	
P. " 15, I, II, III, IV, V, VI, VII	
P. " 17	
P. terrestris	

The foregoing three tables show that there is no correlation between sexuality and growth reaction of these organisms in the presence of minute quantities of crystal violet and malachite green. They also show that, generally speaking, *Phytophthora parasitica* type strains are more resistant to dyes than *P. faberi* or *P. palmivora* types. However, it should be borne in mind that these two types are often interchangeable, whereby *P. faberi* type may dissociate into *P. parasitica* type or *vice versa.*

DISCUSSION

A review of the observations by other workers as well as of the results shown in this paper leads to the conclusion that heterothallism, rather than any biochemical stimuli due to associations, is to be considered the chief factor involved in the production of oospores in the *Phytophthora omnivora* group. The chemical-stimulus hypothesis seems rather far fetched, and Narasimhan is correct in stating that "this view does not explain why oospores are produced only when certain strains are paired" and no others; nor does it explain the behavior of sexually neutral strains, either temporary or permanent. Lester-Smith's theory concerning "a low rate of metabolism characterized by low water content and different ratio of food materials" should be operative just as well when only male strains are paired together or when only female strains are grown in mixed culture. One should be able to obtain oospores by pairing other species of Phytophthora, and species of other genera with either the antheridial or oogonial strains of these heterothallic fungi. Until this is done or until oospores are produced readily and abundantly by the mere manipulation of the environmental conditions, heterothallism will remain the only logical explanation. However, the writer does not believe that the heterothallic

strains discussed in this paper are strictly unisexual. The potentialities of both sexes may be present in some or all of these strains. One sex, however, is so much more dominant and the other so deeply submerged that the different strains behave as unisexual organisms. Just as numerous other characteristics may be carried in the protoplasm for a long time without being manifested and may only occasionally reassert themselves by sectoring away from the old colony, so, the opposite sex may be crowded away and inactivated. The curious periodicity in the oospore production of *P. infestans* and other species strengthens this view. Similarly, the behavior of the inconstant strains discussed in this paper, and the apparent sexual neutrality, even when the opposite sexes are in intimate contact, seem to indicate that sexuality is not a definitely fixed character but that it may depend on both internal and external environmental factors as well as on the introduction of the opposite strains.

Once the presence of heterothallism is definitely established, the ardent taxonomist is going to be confronted by a most disconcerting situation because then many of his so-called species will begin to totter and fall. It is going to make no difference whether we call the phenomenon heterothallism or hybridization, the ultimate result will be the relegation into discard of a number of species that have cluttered the literature. Narasimhan (11) states that "in the case of oospore formation induced by the union of *P. arecae* with *P. parasitica,* it would appear more probable that we have to do with hybridization." When two cultures of Phytophthora, each possessing some more or less definite morphological distinction, unite to give rise to oogonia, such a union is more likely to be considered as true hybridization between two species rather than as heterothallism between male and female strains of the same species. A glance at figures 4 and 5 is sufficient to substantiate such a consideration. When a strain, the largest sporangia of which average not more than 45 μ, mates with another strain whose sporangia average 85 or 90 μ, the orthodox morphologist or the less experienced observer will invariably subscribe to the hybridization theory. It is, however, a well-known but poorly appreciated fact that sporangia are extremely unstable in their morphology and exhibit startlingly radical modifications when the parent cultures dissociate into variants. It is, indeed, very unfortunate that the significance of dissociations in taxonomy is not fully appreciated and is lightly waived aside by Ashby and others by the statement that an avoidance of the use of the kinds of nutrient media which the writer has employed in his work will do away with such dissociations. Artificial suppression of some potential characteristic of a fungus is not going to assure us that such potentialities will not constantly manifest themselves under the more complex natural conditions. We should, on the contrary, stimulate all efforts towards the tracing of the

full sphere of variability of any given organism. Otherwise, every dissociant that may appear in nature will be considered a new species or, at least, a new variety. Even if for the sake of upholding the sanctity of a hollow name we were to assume that *Phytophthora palmivora, P. faberi, P. parasitica, P. terrestris* Sherb., *P. nicotianae* B. de H., *P. manoana, P. meadii,* and *P. arecae* are all true species, and when crossed in cultures the resulting oospores are true hybrids, we would still be unable to get away from the great taxonomic tangle that would result when such oospores germinate to give rise to their progenies. What would then be a pure species, what would be a hybrid, and what a new species? On what ground would they be separated and identified and who would attempt such a task? If we were to admit that any of the organisms listed in the foregoing table are *bona fide* species of one kind and that any other organism that will mate with any of the 48 cultures listed here may be another good species, then any one who happens to isolate a given strain of Phytophthora would have the dilemma of a possible hybrid staring him in the face. Any normal fluctuation or any dissociative phenomena would appear like segregations of hybrid characters, and attempts properly to classify such an organism would resolve into a hopeless task.

No two of the foregoing 48 organisms are identical. In growth habit, in the nature of submerged hyphae, and in the size and shape of sporangia, not to mention host relationships and numerous physiological dissimilarities, these organisms show some striking differences. What sort of progenies will result from the union of such strains? Since mating occurs with the greatest of ease under laboratory conditions, there is no reason to suppose that the same thing will not take place in nature. The extreme variability manifested by the *Phytophthora omnivora* group can be traced directly to this ready-mating habit. The writer has always maintained that such unstable characters as size and shape of sporangia are not of sufficient specific value and that the specific unit can be fashioned only from the combination of both morphological and physiological characters of not only one but of dozens of "type species." But even such a practice will lead us astray unless we be willing to allow a wide margin of variability and to build our species concept on a generously flexible basis. Otherwise, if we insist on looking for some minor and even major differences between the different strains and on elevating such differences to the rank of specific characters, then there will be a never-ending confusion. No two organisms are absolutely alike, not even when they originate from the same spore. There are too many cases showing that single-spore cultures give rise to amazingly different dissociants. A more tolerant spirit in taxonomy is the only guide to freedom from the rapidly complicating mycological catacombs.

SUMMARY

1. Some 85 cultures of *Phytophthora omnivora* were tested. It was found that 48 of these were heterothallic, equally divided into males and females, while the remainder were classified into inconstant forms that showed sexual reactions at one time and no reaction at other times, homothallic forms requiring no other strain for the production of their oospores, and neutral forms showing no reaction no matter in what combinations they were mated.

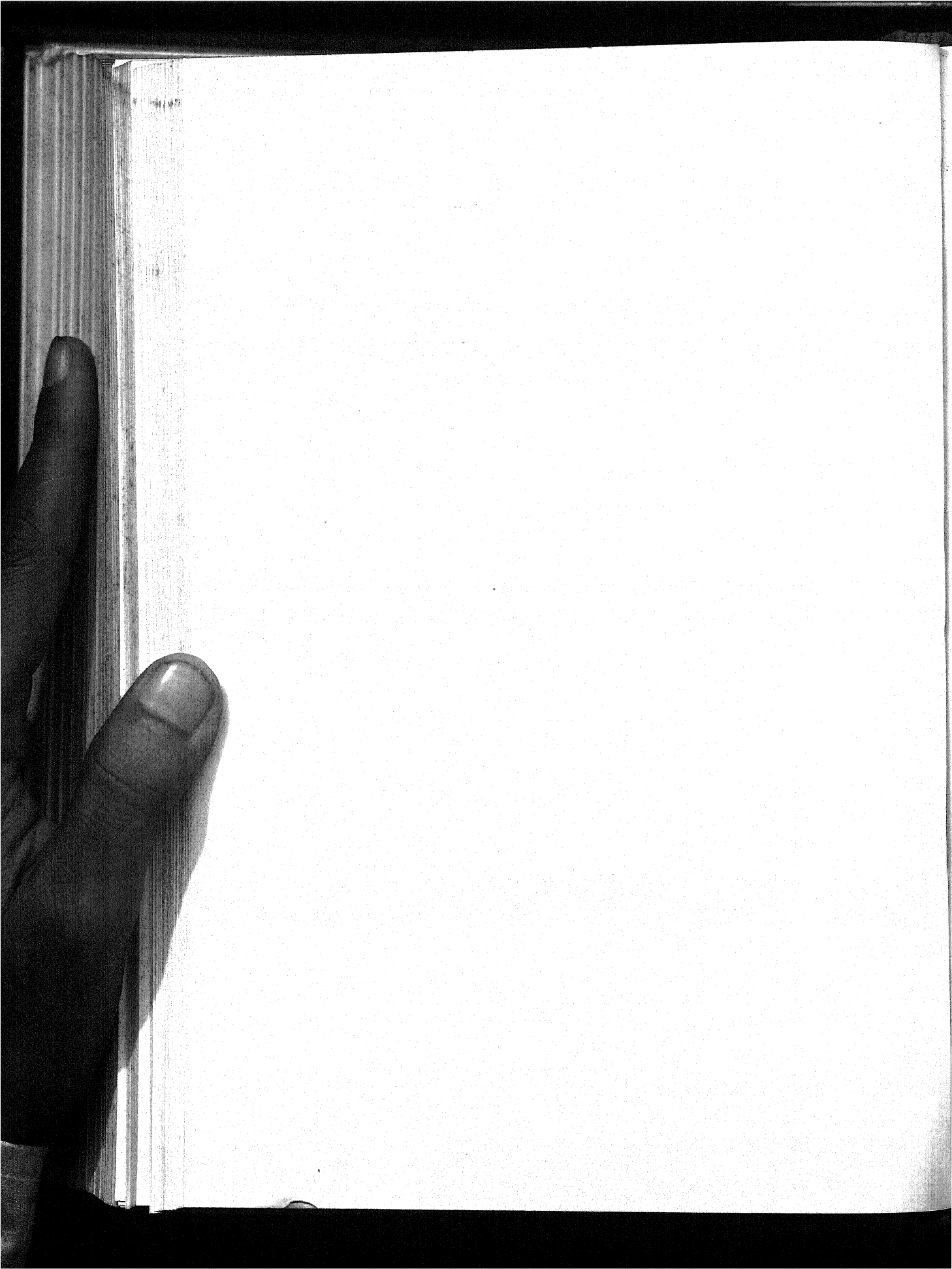
2. Once produced, oogonia cannot be perpetuated indefinitely through the continual association of the two mating forms, and in about five generations no such bodies may form, despite the presence of both strains. Even in the same colony the male and the female strains may separate from each other, despite the most favorable conditions for copulation.

3. There is no correlation between sexuality and ability to tolerate certain concentrations of dyes.

4. None of the 48 strains should be considered a distinct species or even a variety. The phenomenon here exhibited is heterothallism and not hybridization. To admit the possibility of hybridization is to go towards greater taxonomic difficulties.

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REPORT OF THE FOURTH ANNUAL COTTON-ROOT-ROT CONFERENCE

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The fourth annual conference of workers who are engaged in the study of the cotton root rot caused by *Phymatotrichum omnivorum* (Shear) Duggar was held at College Station, Texas, on January 19 and 20, 1931. This conference, which is part of the cooperative attack on the root-rot problem by the United States Department of Agriculture and the Texas Agricultural Experiment Station, affords a yearly opportunity for the prompt presentation of results secured during the previous year at the many laboratories and field stations where work on the problem is under way. The 46 papers presented at this conference included results from 6 laboratories, as well as field and plat studies from 8 stations. A total of 34 plant pathologists, soil chemists, agronomists, botanists, and horticulturists took part in the discussions. Of this group, 18 devote full time to work with root rot, while the remainder are connected with it on a part-time basis or in an administrative or advisory capacity.

Director A. B. Conner of the Texas station and Dr. Oswald Schreiner of the United States Department of Agriculture presided at the various sessions. Following an address by Director Conner on the purposes of the conference, the reports of experimental work were presented; these are summarized below in their order on the program.

LIFE HISTORY AND RELATED STUDIES

Physiologic specialization of the fungus.—D. C. Neal, Bureau of Plant Industry, reported that 6 isolations of *Phymatotrichum omnivorum* from Texas material showed negligible differences in cultural characteristics on artificial media or in soil cultures, while 1 isolation from Arizona was consistently light-yellow to pale-yellow instead of ochraceous to buff. One isolation, apparently attenuated after prolonged growth on artificial media, lost the capacity to produce strand hyphae or sclerotia and yielded only fine white strands and fluffy mycelial growth. Study of the reactions of the various strains to temperature, moisture, and acid or alkali tolerance, showed that optimum conditions for growth were similar for the entire group.

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Physiologic forms differing widely in cultural characteristics were described by W. N. Ezekiel and J. J. Taubenhaus, Texas station. On potato-dextrose agar and synthetic media, those strains assigned tentatively to form 1 produce abundant aerial growth, typical buff strands, and sclerotia, while strains belonging to form 4 grow more sparsely, remain white even in old cultures, produce few strands with typical acicular branches, and do not produce sclerotia on any substrata yet tried. Forms 2 and 3 have intermediate characteristics. The forms have retained their distinctive characteristics in culture for 2 years. They have not yet been tested for possible differences in pathogenicity. Simultaneous inoculations of flask cultures with all possible combinations of 5 of the strains have failed to reveal any evidence of heterothallism in the fungus or any suggestion of the nature of its possible perfect stage.

Sclerotia and strands.—Neal reported that in soil of pH 6.8–7.0, collected in the Mississippi Delta, inoculation with the fungus resulted in good growth and sclerotia production, suggesting that this disease might prove serious in the Mississippi Valley Cotton Belt, if once introduced. Corroborating previous results (3), the optimum moisture requirement for sclerotia production in Wilson-clay-soil cultures was found to be between 30 and 35 per cent (dry basis). The average time for sclerotia formation, with the isolations studied, was 13 days. In soil cultures in the laboratory, approximately 95 per cent of sclerotia were still viable after 13 months, and a few after 22 months. In bottles buried, respectively, 24 and 30 inches deep in the field, no viable sclerotia were found after 8 and 9 months. Sclerotia exposed in covered Petri dishes and in calcium chloride desiccators, at room temperature (24° C.), were viable after 3¼ hours but not after 4 hours. Exposure of sclerotia buried in moist, Wilson-clay soil in small boxes, to electric currents of 250 milliamp.–93 volts, and of 1 amp.–278 volts, a.c., respectively, for 5, 10, and 15 minutes, did not affect subsequent germination of sclerotia (6, 7).

Taubenhaus and Ezekiel described laboratory experiments in which sclerotia were produced in soil chambers from naturally infected cotton-root inoculum brought in from the field at monthly intervals from January through December. In a field plat, previously free of root rot, sclerotia were found as early as August 10, coincident with the death of plants inoculated only 39 days before. Sclerotia were found (9) in the field in 13 counties of Texas; in soils of 14 different types; and in fields of infected cotton, sweet potatoes, garden and sugar beets, carrots, okra, and figs.

B. F. Dana, Texas station, found in field plats at Temple, Texas, viable sclerotia that had survived a fallow period of more than 2 years. Strand sclerotia, which persisted in soil fallowed for 18 months after a cotton crop, were found most abundantly at depths of 12–18 inches. Strand sclerotia

from the field were used in successful inoculations of cotton plants. Sclerotia from the field survived air drying in the laboratory for 49 days in soil that dried to 8 per cent moisture by the end of the period, but sclerotia from the same lot did not survive 7 days' drying on filter paper. S. E. Wolff, Texas station, found large numbers of sclerotia in an excavation in virgin prairie (Houston black clay soil), at 3–21 inches deep, but the majority at 4 inches deep, and associated with the roots of infected, perennial, dicotyledonous plants. No infection was found on grass roots. He observed spore mats in the prairie also.

In experiments on the effect of cold on longevity of the fungus, Ezekiel and Taubenhaus used flask cultures containing masses of sclerotia that had been produced therein, agar slants that had just been seeded with individual sclerotia, and young cultures derived from sclerotia. The cultures were stored in a freezing compartment at $-13°$ to $-14°$ C., and periodic transfers showed that both the sclerotia and the cultures were still viable after 24 hours but not after 39 hours. In similar series at 5° to 6° C., there was no loss of viability after 50 days.

Spore stages.—Taubenhaus and Ezekiel reported that inoculations of susceptible plants with the Phymatotrichum spores proved unsuccessful during 1930, as previously. A Hydnum developed profusely not only along the sides of holes dug near cotton plants affected with root rot but also in holes dug in an area of the field where root rot had not been found for at least 5 years. Cotton plants were inoculated with masses of the spines containing many basidiospores, and with cultures, but no infection resulted (9), suggesting that this Hydnum is probably not a stage of *Phymatotrichum omnivorum*. Dana noted the abundant development of the Phymatotrichum stage in Houston, Catalpa, and Crawford soil areas. Most of the spore mats were found on freshly cut banks of newly graded roads, but exposure to full sunlight did not prevent development so long as the surface of the ground remained moist.

Studies on nutritional requirements of Phymatotrichum omnivorum in artificial culture were presented by Ezekiel, Taubenhaus, and J. F. Fudge, Texas station. The fungus grows in synthetic media, even the sclerotial stage developing in cultures in which the source of nitrogen was ammonium nitrate and the source of carbon was dextrose. At a temperature of 28–29° C., growth curves reached a peak in 5 weeks with a substratum high in dextrose and in 3 weeks with one of lower dextrose content, the media becoming increasingly acid as colonies increased in weight but tending toward alkalinity with later degeneration of the mycelium. In media adjusted with phosphoric acid and potassium hydroxide, respectively, growth was prevented at pH 3 but not at pH 9, the greatest growth occurring in the somewhat alkaline substrata. Requirements for growth are a source of nitrogen,

which may be either organic or inorganic, a source of carbon, phosphate, potassium and magnesium or calcium, and possibly minute amounts of other materials (5).

Host plants.—Wolff and Dana reported as additions to the host list 32 noncultivated species and 35 species and varieties of ornamental plants. Examination at biweekly intervals of representative large populations of some winter annual plants, taken from known root-rot areas, showed 32 per cent infection on *Sitilias multicaulis* Greene on May 3, 1930, and 70 per cent on May 16, 4 per cent infection of *Hamosa Nuttalliana* Rydb. on May 16, but none on *Vicia leavenworthii* Torr. & Gray. Ezekiel and Taubenhaus reported that the guayule, *Parthenium argentatum* Gray an American rubber plant, is susceptible to root rot.

Taubenhaus and Ezekiel (8) summarized inoculation experiments with many monocotyledons, including corn and numerous liliaceous plants, none of which became infected, although the interplanted cotton, okra, and carrot plants succumbed to root rot. Black lesions on the roots of many of the grass plants were cultured and invariably yielded other organisms instead of *Phymatotrichum omnivorum.*

Further studies on the physiologic basis of resistance (5) were given by Ezekiel, Taubenhaus, and Fudge. *Phymatotrichum omnivorum* cultures averaged 5 times as much dry weight of mycelium in nondiluted, autoclaved extracts from roots of cotton plants (susceptible) as in corresponding extracts from roots of corn plants (resistant), and the fungus growth was better also in various dilutions of cotton extracts than in dilutions of corn extracts.

Some effects of root rot on host plants.—Ezekiel and Taubenhaus reported that leaves of cotton plants with root rot were 1° to 6° F., averaging 3° F., warmer than those of normal plants. Normal leaves were usually cooler than air temperature, while leaves of plants affected with root rot were only slightly cooler or often warmer than air temperature (4). Periodic analyses of roots of cotton plants following infection with root rot showed decrease in the percentage content of sugars, with progressive increase in water-insoluble proteins.

Overwintering on live infected roots and cyclic periodicity of root rot.—Taubenhaus and Ezekiel reported successful inoculations of plants in July, 1930, with naturally infected, overwintered 1929 cotton roots, and discussed the relation of overwintering of root rot on roots to the cyclic periodicity of the disease under field conditions (9). The fungus was found on the roots of plants during certain years, even though the tops of all the plants appeared normal.

H. C. McNamara, Bureau of Plant Industry, described the reappearance of root rot in continuous cotton fields, in which the disease apparently had died out, since no plants had died there during the previous 4 years.

Dana and H. E. Rea, Texas station, found that the date of appearance of root rot and the loss from the disease were affected only slightly by differences in the date of planting cotton (2).

STUDIES RELATING TO CONTROL

Occurrence of root rot as related to soil conditions.—Taubenhaus and Ezekiel pointed out that root rot has now been found in soils of 30 series (9) and can no longer be considered peculiar to the Houston black clay soils. Root rot has not been found in the field on plants growing in definitely acid soils.

Progress of studies by the Bureau of Chemistry and Soils on the chemical characteristics of the soils of central Texas was outlined by Paul R. Dawson and E. R. Collins. Complete analyses have been made of a large number of soils. No outstanding chemical factor universally correlated with the presence or absence of root rot has as yet been disclosed; however, in most instances, heavily infested areas are calcareous and alkaline (pH 7–8.3), while root rot is less prevalent or absent in noncalcareous soils of neutral or slightly acid reaction (down to pH 6.0).

Dana and Henry Dunlavy, Texas station, discussed local variations in the soil as related to natural occurrence of root rot. A deep Houston black clay soil, averaging 30.5 per cent soil moisture, appeared more favorable for root rot than a shallower Houston clay, which averaged only 21.2 per cent during the same period. Along a sloping field, of which the flat upper part is Houston black clay, while the main slope is eroded to Houston clay, less root rot occurred in each of 3 years in the Houston-clay part of the field, in which the soil is lower in colloidal content, organic matter, and moisture-holding capacity.

Taubenhaus, Ezekiel, and Fudge reported that continuation during 1930 of the studies on the relation of the soil reaction to incidence and overwintering of root rot gave results agreeing with previous reports (3). Root rot was worse on cotton plants grown in neutral and alkaline soils, with less damage in soils below pH 6.3, and little or none in soil at pH 5.6 or lower. Preliminary studies with crops indicated good growth with cotton, for instance, in soil acid enough to impede the development of root rot. Fudge summarized some chemical studies on the nature of basicity in soils.

Subsoiling experiments, conducted independently by a number of workers, again yielded promising results. Dawson and McNamara reported that in a field planted, in 1929, to sorghum, a plat was subsoiled twice during the dry weather of August; cotton was grown in this field in 1930 with only 1.5 per cent root rot in the subsoiled part of the field, which was separated by a sharp line of demarcation from the control, nonsubsoiled area in which 27 per cent of the plants succumbed to root rot. Neal found a reduction in visible symptoms of the disease in subsoiled plats in 4 different localities

but emphasized the presence of deep-seated infection on the roots of many of the plants. Dunlavy and Dana conducted subsoiling tests on 4 acres and found a reduction of root rot in each subsoiled plat, the reductions amounting to 6 per cent to 48 per cent of the root rot in the respective check plats. There were no consistent differences in yield.

Fertilizers.—Dawson and Howard V. Jordan presented the results of the Bureau of Chemistry and Soils fertilizer experiments conducted in the blackland region of central Texas. As in 1929, increases in yield were obtained from applications of mixtures containing combinations of phosphate and nitrogen. In a number of cases, the increases were profitable from an economic standpoint and in most cases were of such magnitude as to offset losses in yield due to root rot. The more highly phosphatic fertilizers accelerated maturity, thus increasing the yield of the early pickings and suggesting a promising means of evading losses from root rot by such additions. In tests by Rea, Dana, and Dunlavy, some increases in yield were secured by the use of fertilizers at the rate of 400 lbs. or 600 lbs. per acre. Ezekiel and Taubenhaus found that fertilizers applied, in container experiments, at the rate of a ton to the acre produced marked responses in the growth and yield of cotton but did not reduce the incidence or overwintering of root rot. Neal found similarly, in field experiments, that applications of 600 to 1,800 lbs. of fertilizers per acre accelerated maturity of plants and gave increased yields over check plats, tending to counterbalance losses from root rot; however, the amount of root rot was not reduced.

Soil disinfectants.—Neal tested various toxic agents at concentrations of 1:200, immersing sclerotia for 5 to 60 min. In the order of their toxicity, the materials tested were: mercuric chloride, ethyl mercury chloride, chlorophenol nitrophenol mercury, Semesan, copper sulphate, calcium chlorate, and Chlorazene. Additions of 5 per cent of marcasite (crystalline ferric sulphide) to the soil in soil cultures inhibited sclerotia formation; and marcasite applied in field plats at the rate of 7.4 tons per acre apparently reduced root rot.

Comparisons of fungicides in the laboratory by the soil-chamber method previously described (3) were summarized by Ezekiel and Taubenhaus. Among the most toxic materials tested were mercuric chloride, which prevented growth of the fungus at a concentration of 50 ppm. of soil, and Semesan, which inhibited growth at somewhat more than 100 ppm.; while some of the least toxic were aluminum sulphate, copper carbonate, and oxymethylene, which did not greatly affect growth, even at 1,000 ppm.

Dana found that dilutions of 1:80 of sodium, potassium, and calcium chlorates prevented the germination of sclerotia, while dilutions of 1:800 reduced but did not prevent germination.

Field-plat comparisons of a number of disinfectants were presented by Taubenhaus and Ezekiel. The relative quantities of the different materials

needed to reduce incidence and spread of root rot in the field correlated fairly well with the quantities required to inhibit growth in the soil-chamber tests. Some of the organic mercury compounds, such as Semesan, yielded somewhat promising results, while with aluminum sulphate, copper carbonate, manganese sulphate, and iron sulphate, the quantities used produced no apparent effect. W. J. Bach reported that copper sulphate at the rate of 1 lb. per 10 gal. of water, applied around the roots of woody plants, such as grapes, 3 times a year, continued to give good results.

C. J. King, Bureau of Plant Industry, reported that at Indio, California, 1¼ per cent formalin was injected by pressure into the sandy soil to a depth of 6 ft., at the rate of 6 gal. per sq. ft. Prior to the treatment, cultures of the fungus on fig and pistache roots had been placed at various depths in the soil. After 3 to 4 weeks, the fungus grew out again from the interior of roots ¾ of an inch or more in diameter when these roots were placed in moist chambers, but did not grow out from the smaller roots. In laboratory experiments, longer treatments were necessary to kill the fungus inside of roots than to kill sclerotia.

Rotation and cultivation.—McNamara reported that root rot was greatly reduced in cotton following 2 years of sorghum or 2 or 3 years of clean fallow, as compared with plats in continuous cotton or in clean fallow for a single season. Dunlavy and Dana found that the amount of root rot in cotton grown in 2- and 3-year rotations did not differ significantly from that in plats in continuous cotton. Rea summarized tillage experiments in which the treated plats were bedded and rebedded weekly for periods, respectively, of 12 and 24 months. In some of these tests perennial weeds were not eliminated even after 24 months of tillage. These tillage operations, to the shallow depth of 6 in., did not eliminate root rot, and there were significant reductions in root rot in only a few instances.

Barriers to limit the spread of root rot were discussed by Taubenhaus and Ezekiel, with particular reference to some experiments in plats and in containers. Barriers 6 in. wide and 4 ft. deep, consisting of soil into which 4 per cent sulphur had been incorporated, served to check completely the spread of root rot across plats of cotton. Barriers 1 row wide of crops such as sorghum and corn limited root rot to the inoculated rows of cotton plants, in experimental containers, no spread of the fungus occurring either on the roots of these barrier crops or through the soil to reach cotton plants on the other side of the barriers (9).

McNamara reported that of the barriers tested at Greenville, Texas, under field conditions, open trenches 24 in. deep, proved effective in preventing the advance of the disease and sorghum barriers broke up the uniform advance of the fungus.

Flooding experiments at Iowa Park were reported by Taubenhaus and Ezekiel. Although laboratory experiments indicate that the vegetative

stage of the fungus is no longer viable after submergence in saturated soil for 3 days, flooding field plats for as long as 120 days did not eradicate root rot (10).

Resistant strains and varieties.—Bach found that the Champanel, Mustang, Black Spanish, and *Vitis Chantini* grapes retained their resistance to root rot previously reported (3) and that, in addition, the Dog Ridge, *V. Salonis, V. Constancia,* and *V. Berlanderi* grapes appear resistant. The Sour Orange rootstock for citrus still appears highly resistant, inoculations having failed to kill the seedlings. In tests of cover crops for use in citrus groves, *Crotalaria spectabilis* Roth. and *C. striata* DC. and cowpeas proved very susceptible, while *C. incana* L. was somewhat resistant and Sesbania showed sufficient resistance to make it of practical value as a winter cover crop.

With the Turk's cap hibiscus, hackberry, live oak, and pomegranate, well-established plants are highly resistant to root rot, yet in the seedling stage many of the plants may be attacked and a small percentage killed (Taubenhaus and Ezekiel). The seedling plants that recover appear to do so by developing new roots faster than the disease can destroy them, while the fungus apparently is unable to attack the roots of the older plants (1).

Cotton variety tests at Temple Substation were summarized by Rea, Dana, and Dunlavy. A large number of selections and most of the varieties available in the United States have already been tested. During 1930, new introductions to the test were primarily field selections of isolated healthy plants from diseased spots. This work yielded a limited amount of promising material that is to be retested in subsequent years.

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AN ANTHRACNOSE OF LEDUM CAUSED BY A SPECIES OF ELSINOË[1]

S. M. Zeller and J. W. Deremiah[2]

Along the Pacific Coast of Oregon *Ledum glandulosum* Nutt., an attractive evergreen shrub of the Ericaceae, is affected by a very common and serious leaf spot. The senior writer has observed the disease since the spring of 1927. The many collections taken in May and June of 1927 and 1928 gave no clue to the identity of the causal organism. During the winter of 1929–30, however, we examined some older material in the herbarium of the Oregon State Agricultural College. A species of Elsinoë was found on a leaf spot of Ledum collected by G. K. Van Gundia at Manhattan, Tillamook County, July 19, 1915. This suggested that all of our previous collections had been taken before the maturity of the fungus fructifications. This has proved true in collections taken since the first part of July, 1930.

The disease attacks the leaves, the younger branches, flower pedicels, and sometimes the calyx and capsules. The most common and conspicuous expression of the disease, however, is the leaf spot.

The disease has been observed all along the Oregon coast from Brookings, Curry County, to just south of Seaside, Clatsop County. Since the leaf spot is so common within these limits, it perhaps could be found throughout the entire range of the coastal form of *Ledum glandulosum*, and the slight variation of it which Piper has described as *L. columbianum*. On *L. groenlandicum* one specimen from Michigan and one from Washington have been examined.

It is evident that the infection of new leaves is influenced by weather conditions. In situations where fogs frequently are driven in from the ocean the leaf spot makes its first appearance as early as June, but, farther inland, where Ledum is found in bogs at slightly higher elevations, infections on leaves of the current season's growth may not be found until August. On high bluffs overlooking the ocean the spotting is not evident so soon as in lower locations nearby. The disease, however, seems to be as severe on the older foliage in the one location as in the other.

Usually the beauty of the host plant is destroyed by the disease of the leaves and upper branches (Fig. 1, A; Fig. 2, A and B). The size of the leaves differs so greatly that the number of lesions per leaf may not indicate the extent of injury. Some small leaves with a dozen spots may have

[1] Published with the approval of the Director as Technical Paper No. 137 of the Oregon Agricultural Experiment Station.

[2] Mr. Deremiah did the histological work connected with this study.

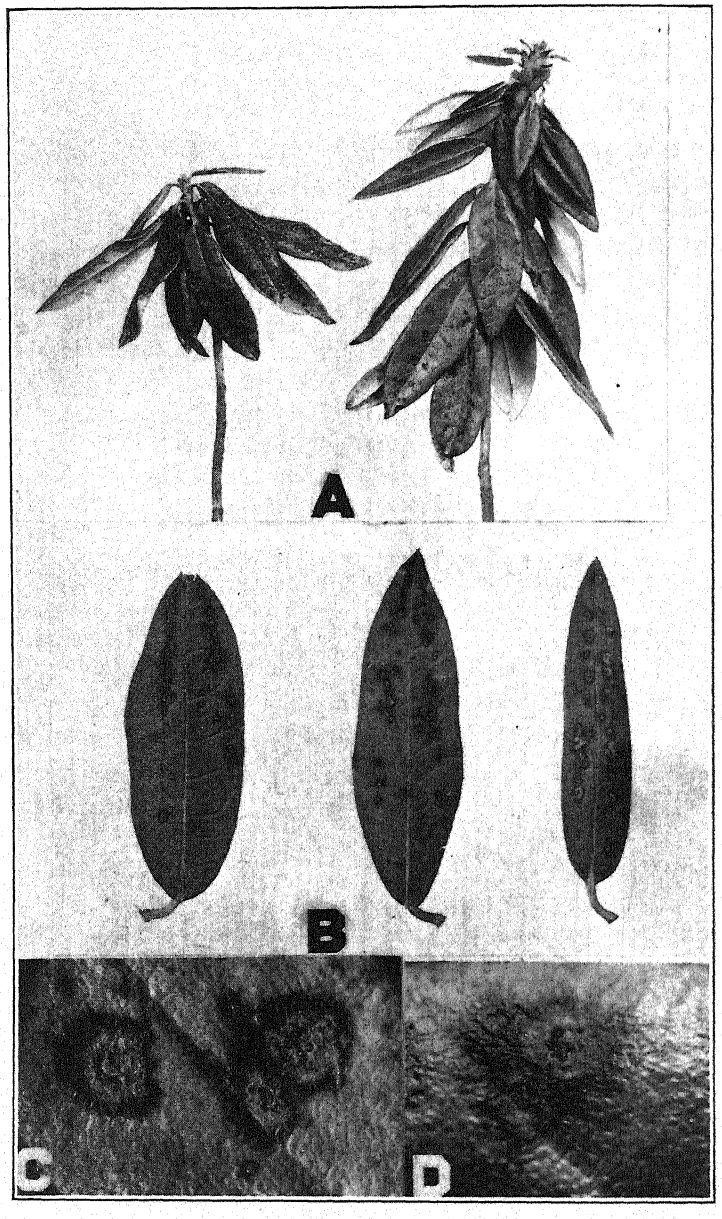


FIG. 1. A. Habit of the branches of *Ledum glandulosum* showing leaves infected by *Elsinoë ledi*. ×2/3. Photo by H. H. Millsap. B. Three leaves showing the characteristic distribution of the leaf spot. ×1. Photo by H. H. Millsap. C. Leaf spots enlarged. ×10. Photo by J. W. Deremiah. D. Leaf spot enlarged to show the fruiting bodies (ascocarps). ×10. Photo by J. W. Deremiah.

50 per cent of the leaf surface destroyed, while another with 35 spots may have two thirds of its surface in apparently healthy condition. From 0 to 47 spots per leaf have been counted (Fig. 1, B).

In some locations the disease causes severe "leaf drop," while, at the same time, in other locations where the spot seems equally severe, there may be no drop. No explanation for this difference is ventured, unless the one location may be drier than the other; but the factors which constitute "physiological dryness" in the sandy bogs where Ledum grows are not easily estimated.

The leaf spot first appears as a tiny circular reddish brown area on the upper surface of the leaf. Spots can be recognized when only $\frac{1}{4}$ to $\frac{1}{3}$ mm. in diameter. The mature ones are from about 1 to 3 mm. in diameter but, where several infections coalesce, they are much larger. As the lesions mature the central part becomes grayish to almost white, while the borders remain reddish brown often with purplish margins (Fig. 1, C). In the central grayish area are to be found the black fruiting bodies of the fungus which is constantly associated with the disease. These fruiting bodies evidently appear within 8 to 12 weeks after the lesions are first distinguishable (Fig. 1, D).

The fruiting bodies break through the upper epidermis of the diseased portion of the leaf. The rupture of the epidermis is usually star-like, having 3 to 5 rays. The fruit bodies of the fungus may take this astral shape or form a flattened hemisphaeroid or elongated pad of dark stromatic tissue.

Vertical sections through the leaf and the fungus were prepared to show the relation of the two. The development of mycelial invasion of the host tissues from the time of infection has not been followed. In mature lesions, however, the fungus hyphae are primarily intercellular, lining the intercellular spaces of the leaf mesophyll and crowding the palisade cells apart. Where the fungous invasion seems complete in the central portion of the leaf spot the crowding of the mycelium has forced the host cells, and, with additional fungous growth, these cells are almost entirely separated, causing almost complete disintegration of the host tissues. At these centers of concentrated fungous growth the fundament of the fruiting bodies pushes up under the cuticle or in some cases raises the whole epidermis. Plugs or palisades of erect hyphae that are cemented into a prosenchyma of somewhat harder consistency bring about the primary rupture of the surface (Fig 2, C). This first concentrated fungous growth is comparable to the ectostroma of some of the Sphaeriales.[3] The general upward growth during this period of development in the interior of the leaf often carries with it

[3] Ruhland, W. Untersuchungen zu einer Morphologie der stroma-bildenden Sphaeriales. Hedwigia 39: 1–79. 1900.

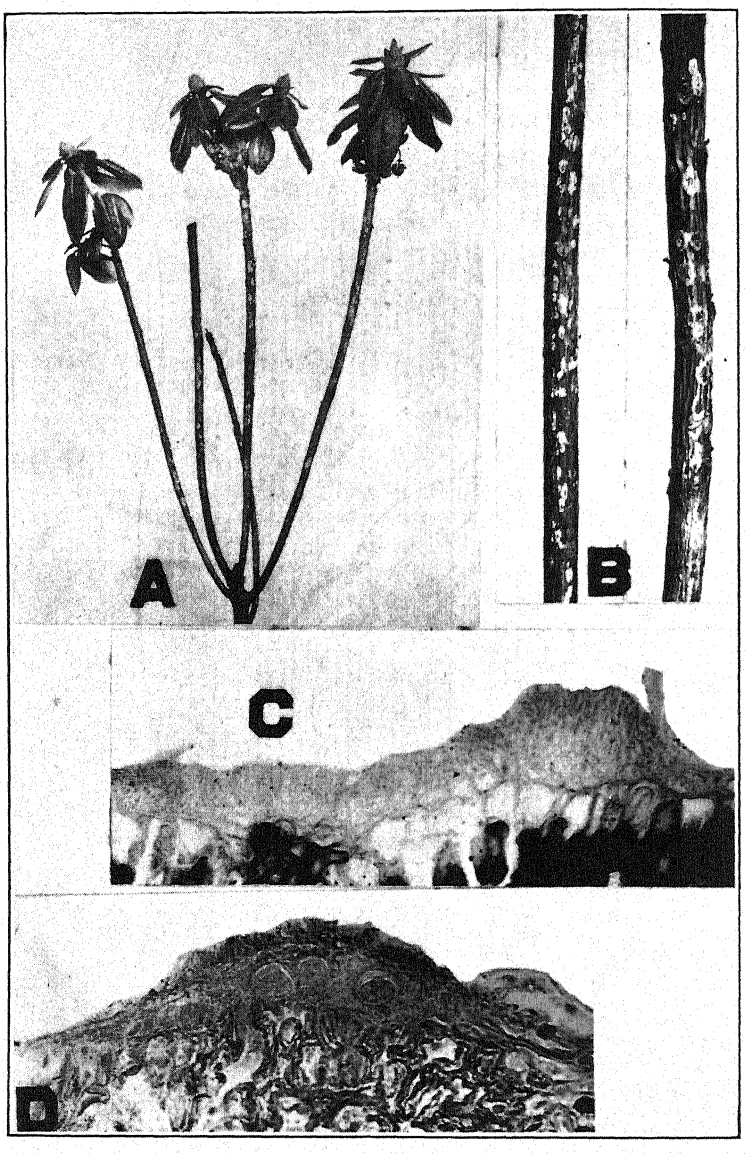


FIG. 2. A. Stems of *Ledum glandulosum* infected by *Elsinoë ledi*. ×2/3. Photo by H. H. Millsap. B. Stems showing anthracnose spots. ×1½. C. Photomicrograph of a vertical section of an acervulus-like, sterile ectostroma showing broken cuticle. The higher darker, raised portion at the right is the palisade of rigid prosenchyma which ruptured the cuticle. The entostroma, or ascocarp, is below and to the left of the ectostroma. ×300. Photo by W. C. Whitaker. D. Photomicrograph of a vertical section of an entostroma (ascocarp) showing three asci and a portion of the ectostroma. ×300. Photo by W. C. Whitaker.

islands of leaf tissue. These may be embodied in the lower part of the mature fruiting body or ascocarp. The mature entostroma or ascocarp is a homogeneous pseudoparenchyma which forms under the original pad of erect hyphae, the latter being sloughed off in a manner closely resembling that in which the acervulus of *Neofabraea malicorticis* (Cordley) Jackson is forced aside by the newly formed ascocarp under it.[4] (Fig. 3.) Some-

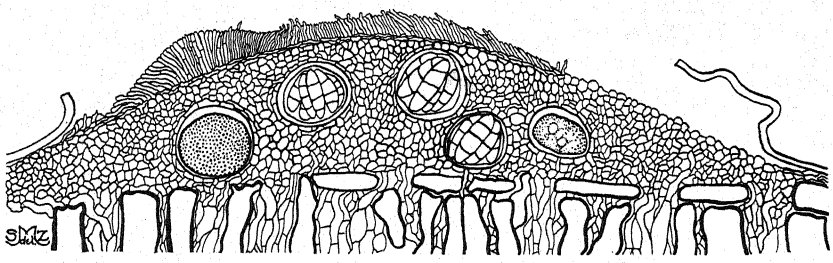


FIG. 3. Drawing showing a vertical section of an entire mature ascocarp illustrating the discarded ectostroma and the ascogenous entostroma. Immature and mature asci usually occur together in an ascocarp. ×500.

times the ectostroma persists more or less loosely attached above or to one side of the mature ascocarp. Even though the ectostroma thus has the appearance and structure of an acervulus, conidia have not been observed in connection with it.[5] The ascocarps occur in various sizes and shapes but the most common semilenticular forms are from 70–195 μ in diameter and have a depth of 28–110 μ. The spherical asci are scattered in monoascoid locules throughout the pseudoparenchyma of the ascocarp. As a rule, the ascogenous locules are near the surface of the structure, more or less in one stratum (Fig. 2, D), but often there is no order of arrangement, the locules being scattered through the pseudoparenchymatous tissue to a depth equaling three to five times the diameter of an ascus. Asci containing 8 spores predominate but those containing 1 to 4 are not uncommon. The asci measure 17–25 x 21–28 μ. The ascospores are ellipsoid to fusoid, often flat on one side, hyaline, mostly 3-septate (seldom 1–2-septate), and measure 12.3–17.7 x 5–6.5 μ.

This fungus belongs to the genus Elsinoë Raciborski, 1900 (Elsinoaceae von Höhnel), which, according to Shear,[6] is synonymous with Plectodiscella,

[4] Jackson, H. S. Apple tree anthracnose. Oreg. Agr. Exp. Sta., Bien. Crop Pest and Hort. Rpt. **1911–1912**: 178–197. 1913. (See Fig. 4, p. 186.)

[5] Since this paper went to press conidia (Sphaceloma) have been found produced by this ectostromatic stage and in a letter Dr. Anna E. Jenkins says leaf lesions from Oregon material show both imperfect stages of the fungus as they are also on the type material.

[6] Shear, C. L. The life history of Sphaceloma ampelinum de Bary. Phytopath. **19**: 673–679. 1929.

Woronichin, 1914. The species found on Ledum is very similar morphologically to *Elsinoë ampelina* (de Bary) Shear, the cause of grape anthracnose, *P. veneta* Burkh., the cause of raspberry and blackberry anthracnose, and also *P. piri,* the cause of a fruit and leaf spot of apple and pear in Europe. The dimensions of the ascospores are similar to those of these species.

The ascocarp, shown in vertical section in figure 2, D, and figure 3, in general, has the regular outline described for *Plectodiscella piri,* but the ascocarp from Ledum is not covered by a sclerotic, placodium-like rind, although the surface is often made up of a darker brown layer of cells with slightly thickened walls. On the other hand, ascocarps of the fungus on Ledum are not all of the type illustrated but are of various shapes, often like the illustrations of those of *P. veneta* or *Elsinoë ampelina,* and are often surmounted by the acervulus-like palisade of the primary ectostroma.

The fungus, constantly associated with the leaf and stem anthracnose reported in this paper, was first described by Peck[7] as *Aulographum ledi.*[8] The description is unmistakably that of the fungus with which we have been dealing, except that in our material the mature ascospores are 3-septate. It was originally discovered on *Ledum groenlandicum* in New York State.

The other species of Elsinoë are on hosts of remote relationship with respect to the Ericaceae. Though the species on Ledum is very similar to the others in morphology, it perhaps should be retained as a separate species until a critical study of the genus is made. The fungus associated with the disease of Ledum is therefore transferred to Elsinoë as **Elsinoë ledi** (Peck) Zeller, n. comb.

The following amended description is based on the material we have examined.

ELSINOË LEDI (Peck) Zeller, n. comb.

Syn. *Aulographum ledi* Peck.

Spots epiphyllous or on stems, circular or coalescing in lines, 1–3 mm. in diameter, at first reddish brown, then whitish gray with reddish brown to purplish margins; *stromata* solitary or gregarious near the center of the spot, circular or irregular, sometimes astral, erumpent, superficially black, pulvinate, 45–200 μ in diameter; *ectostroma* discoid to subconic, of hyaline, erect, palisaded, prosenchyma, rupturing the cuticle or epidermis, bearing

[7] Peck, C. H. Report of the state botanist, 1910. New York State Museum Bul. 150: 23–24. 1911.

[8] We want to express our thanks to Dr. C. L. Shear and Dr. Anna E. Jenkins, who have examined some of the Oregon material and called our attention to *A. ledi* Peck. In a letter under date of February 25, 1931, Dr. Shear says: "An examination of Peck's type shows that it is the same as your Elsinoë."

conidia belonging to the form-genus, Sphaceloma; *entostroma* or ascocarp arising beneath and rupturing the ectostroma which is sloughed off, hyaline interior with brownish surface, pseudoparenchymatous, 70–195 μ in diameter, 28–110 μ high, semi-lenticular to varied in shape, pluriloculate; *locules* subsphaeroid, mostly monoascoid, 19–30 μ in diameter, scattered in the entostroma; *asci* subsphaeroid, 1–8-spored (mostly 4- or 8-spored) hyaline, 17–25 x 21–28 μ; ascospores ellipsoid to fusoid, mostly unilateral, mostly 3-septate (seldom 1–2-septate) 12–17.7 x 5–6.5 μ.

On the leaves, stems, and capsules of *Ledum glandulosum* Nutt., Curry to Clatsop counties, Oregon, and on leaves of *L. groenlandicum* Oeder, Ingham County, Michigan, and King County, Washington.[9]

Specimens examined:

Michigan: Ingham County, Towan's Swamp, East Lansing, May 26, 1895, *A. B. Cordley* (in O. A. C. Herb., 7946).

Washington: King County, north of Seattle, May, 1912, *S. M. Zeller* (in Zeller Herb., 582).

Oregon: Clatsop County, Seaside, July 6, 1928, *S. M. Zeller* & *C. E. Schuster,* (in Zeller Herb., 2199); Coos County, Bandon, June 26, 1928, *S. M. Zeller* (in Zeller Herb., 6774); Lakeside, June 27, 1928, *S. M. Zeller,* (in Zeller Herb., 1759); Marshfield, June 27, 1928, *C. E. Schuster* & *S. M. Zeller,* and Sept. 18, 1930, *L. N. Goodding* (in Zeller Herb., 1668, 7891); Curry County, Brookings, June 24, 1928, *S. M. Zeller* (in Zeller Herb., 6776); Gold Beach, June 26, 1928, *S. M. Zeller,* and July 19, 1930, *L. N. Goodding* (in Zeller Herb., 6775, 7770); Douglas County, June 27, 1928, *C. E. Schuster* & *S. M. Zeller* (in Zeller Herb., 1664); Lane County, Cummins Creek south of Cape Perpetua, June 8, 1929, *S. M. Zeller* (in Zeller Herb., 2664); Lincoln County, Big Creek, January 11, 1931, *S. M. Zeller* (in Zeller Herb., 7882); east of Newport, April, 1927, *S. M. Zeller* (in Zeller Herb., 1765); near Jump-off Joe; north of Newport, Jan. 31, 1931, *Wm. Kessi* (in Zeller Herb., 7896); Waconda Beach, Jan. 11, 1931, *S. M. Zeller* (in Zeller Herb., 7884); near Waldport, July 5, 1930, *S. M. Zeller* (in Zeller Herb., 7769, and O. A. C. Herb., 5548); south of Yaquina John Point, July 30, 1930, *S. M. Zeller* (in Zeller Herb., 7768); Tillamook County, Manhattan, July 19, 1915, *G. K. Van Gundia* (in O. A. C. Herb., 5549).

So far as we are aware *Elsinoë ledi* occurs on Ledum only. There are species of fungi, however, which occur on several different ericaceous hosts.

[9] Dr. C. L. Shear states that there are collections in the U. S. Bureau of Plant Industry on *L. glandulosum* from Mendocino County, Calif., and on *L. groenlandicum* from North Mountain, Pa., Meadowlands, St. Louis County, Minn., and the *type* in the New York State Museum from Fine, St. Lawrence County, N. Y.

One of these, **Cryptostictis arbuti** (Bonar) Zeller, n. comb. (= *Disaeta arbuti* Bonar), frequently occurs in Oregon on *Ledum glandulosum* in close proximity to *E. ledi*. In fact, leaf spots of the two fungi are often found on the same leaf. *Cryptostictis arbuti* was first reported on Arbutus and has been found on Arctostaphylos, and it would not, therefore, be surprising to find *E. ledi* on other ericaceous hosts.

SUMMARY

An anthracnose of the evergreen shrub, *Ledium glandulosum,* is described. It occurs along the Pacific Coast of Oregon and Washington, and one specimen from Michigan on *L. groenlandicum* has been examined. The disease seems to be distributed through the range of the genus Ledum in the United States. Leaf and stem spots are the chief symptoms of the disease. **Elsinoë ledi** (Peck) Zeller, n. comb. (Syn. *Aulographum ledi* Peck) is constantly associated with the disease. The fungus was first described on *L. groenlandicum* from St. Lawrence County, New York. An amended description, with illustrations of the fungus and disease, is included in the present paper.

BLACKLEG OF TOBACCO SEEDLINGS[1]

E. M. JOHNSON AND W. D. VALLEAU

On May 13, 1930, when plants were being pulled for transplanting, the writers found, in a bed of Burley tobacco near Lexington, Kentucky, an area of plants that had a rot which seemed unlike the rots caused by the damping-off fungi. This disease was found in another bed several miles from the first and, later, in several other parts of the first bed. When the cotton was first removed from the bed, the diseased areas were difficult to detect, as the plants, even though almost completely rotted off, were still turgid. The affected areas were found during pulling and could be located without difficulty after the cotton had been off of the bed for some time and after the plants had wilted. The rot started at or near the soil level and extended up the stem 1 to 5 inches, the latter height only rarely. The rotted areas were brown to almost black (Fig. 1), sometimes soft, and the stems often were split longitudinally. Often, the basal leaves were partially to completely rotted. This appeared to be the most usual avenue of entry to the stem. Microscopic examination of affected plants showed the presence of abundant, actively motile, bacteria.

Affected plants, some nearly completely girdled, were set in the field so that the rotted area and some healthy stem tissue came below the soil. All these plants recovered and grew normally. Discolored areas were found



FIG. 1. Tobacco seedlings, transplanting stage, affected with blackleg. Natural infection.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

where healing over occurred, but attempts to isolate bacteria from such areas were not successful.

Bits of affected tissue from seedling plants were removed aseptically, crushed in melted potato-dextrose agar, and plates poured. In from 14 to 20 hours convex, glistening, smooth to amoeboid, translucent, colonies of bacteria developed. Turkish tobacco plants, 8 to 10 inches tall, were inoculated with 48-hour-old potato-dextrose agar tube cultures, from representative colonies, by means of needle stabs half way up the stems. Following inoculation, the plants were placed in moist chambers. Twelve hours after inoculation, water-soaked discolored areas were present around the needle pricks, and in 36 hours the rotting had extended along the stem 2 to 3 inches, above and below the stab. The leaves in the neighborhood of the rotted area drooped, and in some plants the rot extended into the petioles (Fig. 2, A). When removed from the bell jars, some plants broke at the rotted areas. Bacteria reisolated from these plants again caused typical stem rot in Turkish tobacco plants.

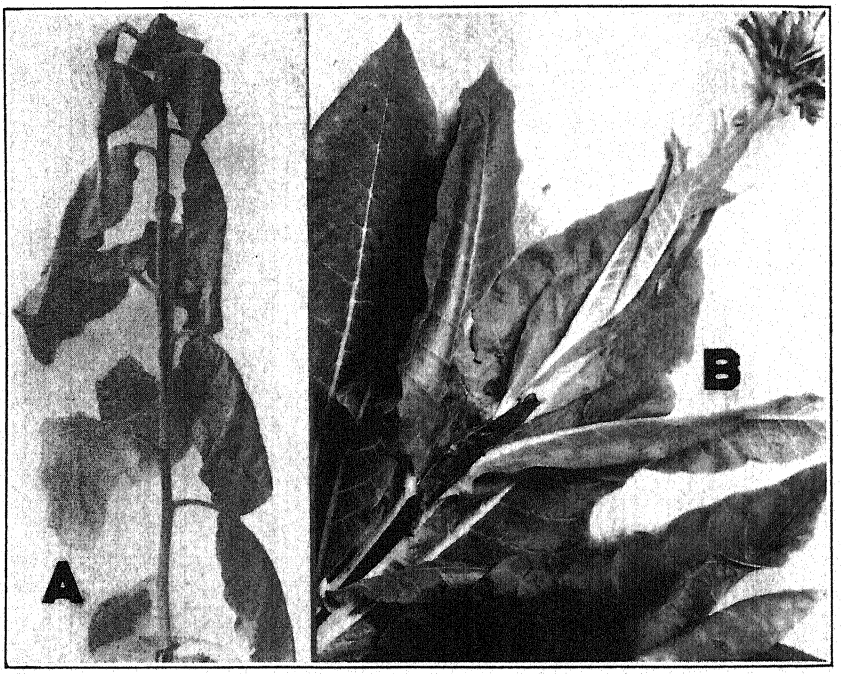


FIG. 2. A. Turkish tobacco plant inoculated with tobacco-blackleg organism. Longitudinal split, drooping leaves, and hollow stalk are shown. B. White Burley tobacco plant inoculated with tobacco-blackleg organism. Bacteria were inserted into a pin prick into the pith 8 inches below the top. Photographed 3 days after inoculation.

Forty-eight-hour-old cultures of the tobacco organism were used to inoculate almost mature White Burley plants in the field. The bacteria were inserted into the pith 8 inches below the tip, near the middle of the stem, and 6 inches from the base of the plants. The stabbed areas were wrapped with moist cotton and paper. After 20 hours there was a brownish, soft, rotted area extending up and down the stem 1½ inches on either side of the stab in the plant inoculated near the top. After 24 hours more the rotted area was 7 inches long and almost girdled the stem. The plants at this time and later were typical of those affected with the disease known as hollow stalk (Fig. 2, B). In the plants inoculated near the middle of the stem and near the soil, the lesions were never longer than 3 inches, and, after 2 weeks, wound tissue formed around these areas. White Burley tobacco plants in the same planting were topped, bacteria pricked into the exposed pith, and moist cotton placed around the inoculated areas. A brown, soft, rotted, area 3 inches long developed in 20 hours. The pith at the top was a soft, slimy, brownish mass with an unpleasant odor. The pith rot seldom extended more than ½ inch down the stem, in contrast with the more extensive rot, when the outer tissues were also infected.

COMPARATIVE STUDIES OF THE TOBACCO ORGANISM WITH OTHER SOFT-ROT ORGANISMS

It was suspected that the organism might belong to the soft-rot group. Through the kindness of J. G. Leach, of the University of Minnesota, three cultures of *Bacillus carotovorus* Jones (I, C, and 3–A) and one of *B. aroideae* Townsend were obtained.[2] Limited comparative cultural, physiological, and pathogenicity studies were made with these and the tobacco organism. All of them hydrolyzed starch, were gram-negative aërobes, and produced filiform, convex, glistening, smooth, and translucent colonies on beef-peptone-agar slants. *Bacillus aroideae* and the tobacco organism clouded beef-peptone broth more than the cultures of *B. carotovorus*. *Bacillus aroideae* and the tobacco organism produced only acid in beef broth containing 1 per cent glucose, sucrose, and lactose, whereas the cultures of *B. carotovorus* produced both acid and gas.

Comparative pathogenicity on potatoes, carrots, and tobacco. The cut ends of carrots, surface-sterilized with 1 to 1,000 bichloride of mercury, were stabbed with a needle dipped into 48-hour-old broth or agar cultures of *Bacillus carotovorus, B. aroideae,* and the tobacco organism. After inoculation the carrots were placed in a damp chamber. *Bacillus aroideae,* the tobacco organism, and one culture of *B. carotovorus* (C) produced soft

[2] The writers wish to thank Dr. Leach for helpful suggestions and the Division of Plant Pathology and Botany of the University of Minnesota, where some of this work was done, for laboratory facilities.

rot of the carrot. Carrots, rotted with *B. aroideae,* and the tobacco organism were similar and had a brown discoloration not present in those affected with *B. carotovorus* (Fig. 3).

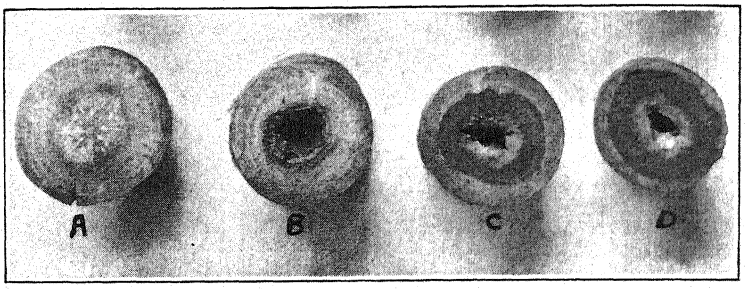


FIG. 3. Cut ends of carrots inoculated with soft-rot bacteria and the tobacco-blackleg organisms. A. Check. Not inoculated. B. *Bacillus carotovorus* (C). C. *B. aroideae.* D. Tobacco-blackleg organism.

Half-inch cylinders were cut from surface-sterilized potato tubers by means of a flamed cork borer. Inoculum from the cultures used to inoculate the carrots was placed on the cut surfaces of the cylinders near the middle, and these were replaced in the holes in the tubers. The latter were placed in a damp chamber. All the cultures of *Bacillus carotovorus* produced some soft rot after 24 hours. Two (I and 3-A) produced only a slight soft rot that did not spread after 48 hours, while the culture (C)

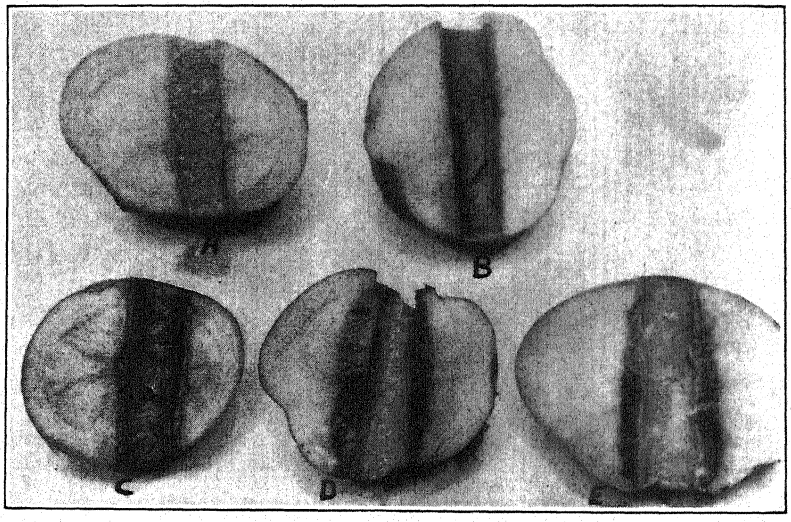


FIG. 4. Potatoes inoculated with soft-rot bacteria and the tobacco-blackleg organism. A. Check. Not inoculated. B. *Bacillus carotovorus* (3a). C. *B. carotovorus* (C). D. *B. aroideae.* E. Tobacco-blackleg organism.

that rotted carrots caused a soft rot extending ⅛ inch into the plug and the same depth into the tuber around the plug. After 24 hours the plugs of tubers inoculated with *Bacillus aroideae* and the tobacco organisms were soft, slimy, masses; and, 24 hours later, the rot had extended into the tuber ½ inch around the plug (Fig. 4).

Two cultures of *Bacillus carotovorus* (C and 3-A), *B. aroideae,* and the tobacco organism produced stem rot of 6-inch Turkish tobacco plants, like that previously described for the tobacco organism.

IDENTITY OF TOBACCO ORGANISM

The tobacco organism resembles *Bacillus aroideae* morphologically, culturally, physiologically, and pathogenetically. Both are peritrichous rods, 2.5 to 3 μ in length by 1 to 2 μ in diameter (Fig. 5). Both react the same on sugar media and differ from *B. carotovorus* in the production of acid but not gas in dextrose, lactose, and sucrose broths. Both produce the same type and degree of rotting on tobacco plants, potato tubers, and carrots. The tobacco organism seems to be one of the soft rot organisms and is probably the same as *B. aroideae* which Massey[3] considers as a separate species from *B. carotovorus.*

DISCUSSION

Bacteria of the soft rot group have been suggested as a cause of hollow stalk of tobacco in the field, but, so far as the writers are aware, injury from these organisms has not been reported in plant beds. The similarity

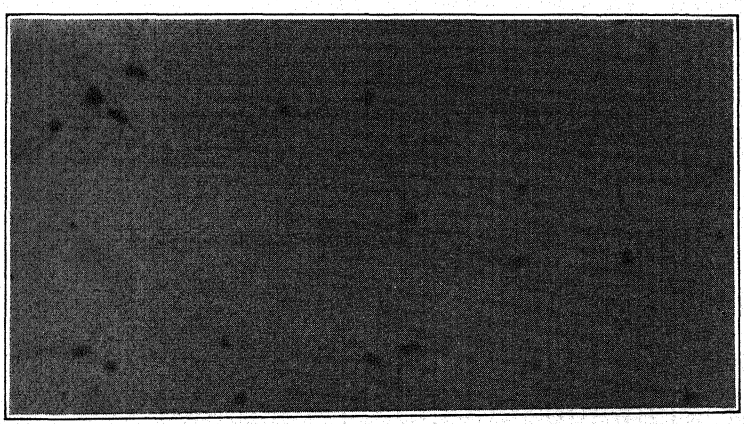


FIG. 5. Tobacco-blackleg organism. Forty-eight-hour-old agar culture. Stained by Casares-Gils method. ×1,000.

[3] Massey, A. B. A study of *Bacillus aroideae* Townsend the cause of a soft rot of tomato, and *B. carotovorus* Jones. Phytopath. **14**: 460–477. 1924.

of symptoms of tobacco plants affected with *Bacillus carotovorus* and *B. aroideae* suggests that either of these may be pathogenic to tobacco and, with favorable conditions, might cause blackleg of seedlings, or hollow stalk of older plants in the field.

The writers observed this disease in a plant bed on the Experiment Station farm at Lexington a few years ago. Discussions with growers indicate that they are familiar with the disease and that they fear it during rainy periods when the plants are large and crowded in the bed.

Leach[4] has demonstrated that the pathogene of potato blackleg, which, in a later paper,[5] he assigns to the soft rot group, can overwinter in the soil in Minnesota. It is probable that soft rot bacteria may inhabit many soils. If temperature and moisture conditions be favorable, soft rot bacteria may enter seedlings through the hydathodes, as it is not unusual to find the rot starting at the tips of leaves that touch the soil. The organism might enter the tobacco plant through insect wounds, but no case of this kind has been observed by the writers. The mode of entry into plants in the field is not known.

SUMMARY

A stem rot of tobacco seedlings at the transplanting stage is described as it was observed in two plant beds in Kentucky. Microscopic examination of affected plants showed the presence of actively motile bacteria. The name, blackleg, is suggested for the disease because of its resemblance to blackleg of potato.

Bacteria isolated from affected plants caused a stem rot of Turkish tobacco in the greenhouse and a stem and pith rot of Burley tobacco in the field. Soft rot of potatoes and carrots was produced by cultures of the stem rot pathogene.

Comparative studies indicate that the stem rot organism is similar to, if not identical with, *Bacillus aroideae* in that it produces acid but not gas on beef broth containing lactose, dextrose, and sucrose.

KENTUCKY AGR. EXP. STATION,
LEXINGTON, KENTUCKY.

[4] Leach, J. G. Potato blackleg: The survival of the pathogene in the soil and some factors influencing infection. Phytopath. 20: 215–228. 1930.

[5] Leach, J. G. The identity of the potato blackleg pathogene. Phytopath. 20: 743–751. 1930.

SEED TRANSMISSION OF COWPEA FUSARIUM WILT

JAMES B. KENDRICK

The wilt of cowpeas (*Vigna sinensis* (L.) Endl.) caused by *Fusarium tracheiphilum* (E.F.Sm.) Wr., is a serious factor in nearly all sections of California where blackeye cowpeas are grown. In many cases, the soil has become so thoroughly infested with the wilt that the growing of blackeye cowpeas is no longer profitable. The increasing severity of the disease has forced many growers to use new areas for growing "blackeyes," as they are commonly known in California. Many complaints have been received of the occurrence of wilt in areas where blackeyes have not been previously grown. Observational evidence pointed to the possibility that the disease was being spread with the seed. In so far as the writer is aware, there is no record to show that this disease is seed-borne. Since the wilt is of major importance in the growing of blackeye cowpeas in California, it seemed advisable to determine experimentally if the agent responsible for the disease was being disseminated with the seed.

The disease was first described by Smith[1] in 1899. In 1902 Orton[2] published a rather comprehensive account of the same disease. As was pointed out by Orton and from three years' observations in California, cowpea wilt does not appear until the plants are about 6 weeks old. At first, a few plants are noticed throughout the field with pale green, flaccid leaves, which soon turn yellow and drop from the plant. The plants showing the disease early in the season usually die prematurely and fail to mature seed. As the season advances, more and more plants show the disease, as evidenced by their dwarfed condition, yellowness, and, in many cases, death of the infected plants. However, not all diseased plants show external symptoms. In experimental plots, where each plant has been pulled at the end of the season and the stem cut for evidence of the disease, many large, apparently healthy plants have been found with a greatly swollen and roughened condition of the lower part of the stem and main root (Fig. 1, A). When the stems of such plants are examined more carefully, the vascular system shows as a dark-brown mass of disintegrated tissue with only the outer cortical area showing evidence of life. The vascular discoloration often extends throughout the plant. Mature plants have been observed at the end of the season, which appeared vigorous and healthy, but, on examination of the root system, they were found to be in a badly dis-

[1] Smith, Erwin F. Wilt disease of cotton, watermelon and cowpea. U. S. Dept. Agr., Div. Veg. Phys. and Path. Bul. 17. 1899.

[2] Orton, W. A. Some diseases of the cowpea. U. S. Dept. Agr., Bul. Plant Indus. Bul. 17. 1902.

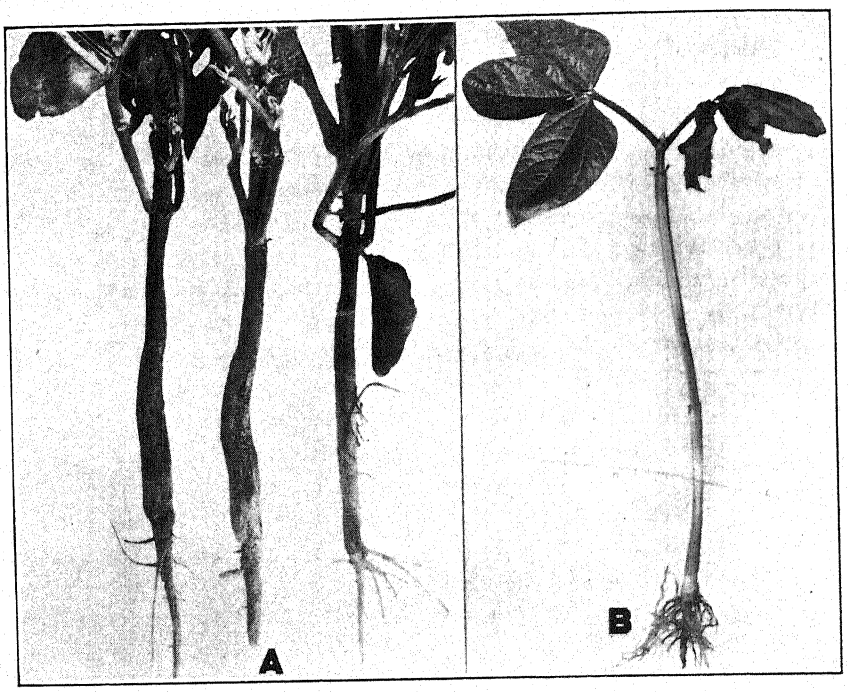


FIG. 1. A. Two blackeye-cowpea plants on the left, severely infected with *Fusarium tracheiphilum*, showing the swollen and roughened condition of the lower stem and main root, while the foliage showed no evidence of disease. Such plants often mature a fair amount of seed and are harvested with the healthy plants. Healthy plant on the right. B. Seedling blackeye cowpea grown in steam-sterilized soil from seed harvested by heating mature diseased plants in a burlap bag. The wilted leaf on one side denotes the first evidence of wilt.

eased condition. Other plants, with a much less severely diseased vascular system, die from the disease.

Many plants that show the disease by midsummer also survive and mature a partial crop of seed even though they lose all or part of their leaves and show severe vascular infection throughout the plant. Since many diseased plants mature seed, the possibility that the fungus responsible for the disease might be carried with the seed seemed likely.

In order to determine whether the fungus penetrated the seed and was harbored as intraseminal mycelium, a series of cultures were made. Macroscopic examination of diseased plants showed the brown discoloration in the vascular system of all parts of the plant. Peduncles with attached ripening pods were removed from severely diseased plants. The pods were carefully plucked and the peduncles surface-sterilized with mercuric chloride 1–1000 and then washed in sterile water. Small cross sections

were cut aseptically from the basal and apical end of each peduncle and planted on potato-dextrose agar. The pods previously removed from the peduncles were carefully opened with sterile tweezers and the first pea from the basal end of each pod was plated on potato-dextrose agar, care being taken to place the hilum in contact with the agar.

Platings were made from the basal end of 205 peduncles, of which 34.6 per cent showed the presence of *Fusarium tracheiphilum*. Out of 271 apical ends of peduncles thus cultured, 4.8 per cent yielded *F. tracheiphilum*, while 315 seeds plated as stated above failed to show any evidence of *F. tracheiphilum*. The pathogenicity of the cultures was determined by greenhouse inoculations in sterile soil. The above results indicate that the causal fungus quite often penetrates the peduncle and in some cases reaches the pod, but in no case was there evidence of penetration of the first seed in the pod. However, more extensive tests may show that the fungus does, in some cases, enter the seed.

Two methods of securing seed for testing were used. In one instance, an attempt was made to simulate harvest conditions as near as possible and, in the other case, ripe pods were carefully removed from severely diseased plants by hand. It may be well to state that the general method used in harvesting beans in California is to cut the plants just beneath the soil by running a cutter along the row. The plants are then raked into windrows and allowed to dry, after which they are run through a threshing machine. Since a great deal of diseased material is thus run through the threshing machine, no doubt abundant spore material is liberated and lodges on the seed coat. While harvesting some experimental plots in 1929, a large number of diseased plants were pulled and placed in a large burlap bag. After the plants were dry, the beans were threshed by beating the plants in the bag and the seed removed from the trash by means of an electric fan. At the same time a quantity of seed was harvested by hand-picking ripe pods from a number of plants showing severe wilt. The 2 lots of seed were kept separate and used for subsequent greenhouse tests. For convenience the 2 lots of seed were designated as threshed seed and hand-picked seed and will be thus referred to in this paper. All seed tests were made in soil that had been steam-sterilized at 40-pounds pressure for 3 hours.

On October 24, 1929, 4 flats were planted with threshed seed and 20 6-inch pots with hand-picked seed. The first evidence of wilt occurred 43 days after planting. As soon as a plant showed evidence of disease (Fig. 1, B), it was removed and cultures made. Final notes were taken on January 21, at which time all plants were removed and the main stem cut for evidence of vascular infection. Many plants showed symptoms more or

less typical of those occurring under field conditions. The results of the above test showed that out of 152 plants grown from threshed seed 40, or 26.3 per cent, developed wilt symptoms, while 147 plants grown from hand-picked seed remained healthy.

Fourteen flats of sterile soil were planted on January 31, 1930, with the same lot of threshed seed used in the previous trial. In this test, 1,028 plants developed, of which 168, or 16.3 per cent, developed wilt.

Since there was a possibility of secondary spread from the early diseased plants to those in close proximity in the two former trials, a third set of plantings was made on April 10, 1930, in which single peas were planted in 4-inch pots of soil. At the same time, 10 peas were planted in each of 31 6-inch pots and 188 6-inch pots were planted with 3 lots of hand-picked seed. The results are presented in tabular form below.

TABLE 1.—*A comparison of seed transmission of cowpea Fusarium wilt where the seeds were harvested by two different methods and grown under different conditions*

Seed lot	Method of growing	Number of plants	Percentage showing wilt
Threshed seed	10 peas in each 6-inch pot	259	8.5
"	1 " 4- "	574	3.9
Hand-picked seed Lot 1	10 " 6- "	210	0
" " 2	10 " 6- "	344	0
" " 3	10 " 6- "	255	3.5

The results presented in table 1 indicate that there may have been some secondary spread, where more than 1 plant was grown in a container. However, since 214 young plants growing in individual 4-inch pots were killed by small root maggots, it is possible that the incidence of the disease may have been increased if these plants had not been destroyed. It will also be noted that in 1 lot of hand-picked seed 3.5 per cent of wilt developed.

In the fall of 1930 2 additional seed lots were collected, using the same methods of harvesting that were used in 1929. On November 5, 1930, 12 flats were planted with 1930-threshed seed and 10 flats with 1930-hand-picked seed. The results showed that of the 574 plants grown from threshed seed 65, or 11.3 per cent, developed wilt, while the 560 plants from hand-picked seed showed 1 with definite wilt symptoms.

The evidence proves quite conclusively that the agent responsible for cowpea wilt is carried on the seed. In 2 out of 5 trials, a small percentage of wilt occurred where plants were grown from hand-picked seed. As to whether this resulted from the organism being inside the seed coat or from

other sources, positive proof is lacking. This point is being investigated further.

In order to determine if the causal agent of cowpea wilt lives from one season to another on the seed, 6 flats of sterile soil were planted on September 12, 1930, with 1-year-old threshed seed. The results showed that out of the 305 plants 22, or 7.2 per cent, showed wilt symptoms. A summary of the seed tests previously made with the same lot of seed shows an apparent reduction in the percentage of wilt as the seed becomes older. Seed 43-days old showed 26.3 per cent wilt; 142-days old, 16.3 per cent wilt; 205-days old, 8.5 per cent wilt; and 1-year old, 7.2 per cent wilt.

Additional evidence that the seed is responsible for the dissemination of cowpea wilt was secured when a commercial lot of seed, suspected of harboring wilt, was tested. A grower used 2 lots of seed to make his 1930 planting. The planting from 1 of these seed lots developed a small percentage of wilt, and circumstances pointed to the seed as the source of the disease. A subsequent greenhouse test in which 320 plants were grown from this seed showed 4, or 1.25 per cent, showing definite wilt symptoms.

SUMMARY

Preliminary trials have failed to show that the fungus (*Fusarium tracheiphilum*), causing cowpea wilt, penetrates the seed. Evidence is presented that the fungus is transmitted on the seed where machine methods are used for harvesting. Hand-picked seed from diseased plants showed a low percentage of wilt in 2 out of 5 trials. It has also been found that a rather high percentage of wilt resulted from using seed 1 year old.

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SEED-TREATMENT AND DATE-OF-SOWING EXPERIMENTS WITH SIX VARIETIES OF FLAX[1]

L. C. BURNETT[2] AND CHAS. S. REDDY[3]

Flaxseed production in Iowa was greater in 1930 than in any year since 1908. There was an increase of 75 per cent in acreage and 110 per cent in production over the 10-year average (1920–1929). In view of this added interest, preliminary experiments were inaugurated to study the influence of date of sowing and of seed treatment upon the stand and yield of flax. These experiments were conducted with the leading wilt-resistant varieties at four places in Iowa.

Yields of 6 varieties are presented in table 1 for the May 1 and May 14 dates of sowing. The sowing on May 31 produced such a poor crop that it was not harvested but was discarded in the field.

TABLE 1.—*Effect of date of sowing on acre yields of 6 varieties of flax at the Agronomy Farm, Ames, Iowa, 1930*

	Bison check	N D R 114	Red-wing	Lin-ota	Buda	Bison	Rio	Mean
Sown May 1								
Acre yield (bu.)	17.72	15.20	16.82	14.55	12.52	18.05	14.27	15.59
Sown May 14								
Acre yield (bu.)	7.92	9.22	12.90	8.10	5.45	7.55	7.12	8.32
Loss from late sowing								
Bushels	9.80	5.98	3.92	6.45	7.07	10.50	7.15	7.29
Percentage	55.3	39.6	23.3	44.4	56.5	58.2	50.2	46.6

The data in table 1 show that satisfactory yields were obtained from the first sowing; little more than half as much (53.4 per cent) from the second sowing; and practically no yield from the third. The differences, no doubt, were accentuated by the exceptionally dry season of 1930.

Thirty observations at Ames on stands and yields showed variations from 119 to 506 plants per 17-ft. row with corresponding yields ranging from 130 to 197 gm. (13.0 to 19.7 bu. per acre). In these data an increase in yield of 1 bushel per acre was obtained with each increase of 1 plant, from 8 to 10 plants per square foot. Above that number (10 per square foot) an increase of about 4 plants per square foot was required to secure an increase of one bushel per acre.

[1] Published with the approval of the Director as a Journal Paper of the Iowa Agricultural Experiment Station, Ames, Iowa.

[2] Chief in Cereal Breeding, Farm Crops Section.

[3] Assistant Chief, Botany and Plant Pathology Section.

Seed-treatment experiments, using seed of Bison flax (96 per cent germination) obtained from Fargo, North Dakota, were also conducted on the Agronomy Farm at Ames (10 replications sown on May 1), on Plant Pathology plots at Ames (10 replications sown on April 28), at Belmond (3 replications sown on April 22), and at Mason City (3 replications sown on April 23). The rate of sowing was 5 gm. or approximately 940 seeds per 17-ft. row. Dust fungicides comprising 2 trial dusts (G-2-2 and 31C), Corona Oat Dust (COD), Ceresan (Cer), and American cyanamid No. 7 (Am. Cy. 7) were used at the rate of 2 ounces per bushel. The stand data were obtained when the plants were mature by pulling, and counting 5 of the replications at the Agronomy Farm, 1 at the Plant Pathology plots, 3 at Belmond, and 3 at Mason City (table 2).

TABLE 2.—*Field stands from nontreated and treated Bison flaxseed sown at four places in Iowa, 1930*

Location of experimental field	Replications	Number of plants per 17-ft. row[a]					
		Non tr.	Treated with:				
		Check	G–2–2	31–C	COD	Cer.	Am. Cy. 7
Ames, Agr. Farm	5	140	271	185	190	401	169
Ames, Path. Plots	1	362	481	455	398	516	390
Belmond	3	502	462	491	451	598	462
Mason City	3	277	449	412	351	406	252
Weighted mean	(12)	283.2	380.6	340.8	312.8	461.1	281.2
Percentage of check							
Ames, Agr. Farm	5	100	193.5	132.1	135.7	286.3	120.7
Ames, Path. Plots	1	100	132.8	125.2	110.0	142.5	107.7
Belmond	3	100	92.1	97.8	89.8	119.2	92.1
Mason City	3	100	162.0	148.7	126.7	146.5	91.0
Weighted mean	(12)	100.0	134.2	120.2	110.3	162.7	99.3

[a] Approximately 940 seeds were planted in each row.

The data in table 2 show that increases in stand were obtained from all the treatments except Am. Cy. 7. Nontreated seed produced percentage stands ranging from 15 to 53 (140 to 502 plants per row). Under the same conditions and with seed of the same lot the percentage stands from the best seed treatment, Ceresan, ranged only from 42 to 63 (401 to 598 plants per row).

The yield data from the seed-treatment plots are presented in table 3, and data from tables 2 and are summarized in table 4.

TABLE 3.—*Yields from nontreated and treated Bison flaxseed sown at four places in Iowa, 1930*

Location of experimental field	No. Replications	Yield in grams per 17-ft. row					
		Non tr. Check	Treated with:				
			G–2–2	31–C	COD	Cer.	Am. Cy. 7
Ames, Agr. Farm	5	145	168	147	148	194	169
Ames, Path. Plots	1	140	200	120	140	195	110
Belmond	3	163	160	162	157	187	149
Mason City	3	183	187	202	188	223	181
Weighted mean	(12)	150.2	173.8	162.2	159.6	199.6	162.1
Percentage of check							
Ames, Agr. Farm	5	100	115.8	101.4	102.0	133.7	116.5
Ames, Path. Plots	1	100	142.9	95.7	100.0	139.2	78.6
Belmond	3	100	98.2	99.4	96.4	114.6	91.4
Mason City	3	100	102.1	110.4	102.7	121.8	99.0
Weighted mean	(12)	100.0	115.6	108.0	106.3	132.9	107.9

TABLE 4.—*Relation of stand to yield in the 12 replications of the seed-treatment experiment conducted on four experimental fields in Iowa in 1930*[a]

Seed treatments	(None) Check	G–2–2	31–C	COD	Cer.	Am. Cy. 7
Stand:						
Plants per 17-ft. row	283.2	380.6	340.8	312.8	461.1	281.2
Percentage of check	100.0	134.6	120.3	110.4	162.7	99.3
Yield:						
Grams per 17-ft. row	150.2	173.8	162.2	159.6	199.6	162.1
Percentage of check	100.0	115.6	108.0	106.3	132.9	107.9

[a] Data from tables 2 and 3.

Table 4 shows (1) that the highest field stand produced the highest yield and that, except for Am. Cy. 7, the yields are directly correlated with the stands; and (2) that all the treatments, except Am. Cy. 7, gave increases in stand and that all the treatments gave increases in yield.

The yield data presented in table 5 for 26 replications include 14 for which no stand counts were made.

Table 5 shows that yields were increased by both G-2-2 and 31-C, but better gains were made following the use of Ceresan.

TABLE 5.—*Acre yields*[a] *from nontreated and treated Bison flaxseed sown at four places in Iowa, 1930*

Location of experimental field	Replications	Nontreated Check	Treated with: G-2-2	31-C	COD	Cer.	Am. Cy. 7
Ames, Agr. Farm	10	15.15	17.95	16.30	13.40	19.75	13.90
Ames, Path. Plots	10	11.55	12.45	12.20	11.20	14.85	11.20
Belmond	3	16.30	16.00	16.20	15.70	18.70	14.90
Mason City	3	18.30	18.70	20.20	18.80	22.30	18.10
Weighted mean	(26)	14.26	15.70	15.16	13.44	18.04	13.46
Percentage check		100.0	110.1	106.30	94.20	126.50	94.4

[a] Calculated from replications of 17-ft. rows.

Ceresan also improved the stands and yields of 5 other varieties in plots of 2 replications each sown on May 14. The effect on stand and yield is presented in table 6, in addition to the data for Bison, which has been brought forward from the foregoing tables.

TABLE 6.—*Effect of Ceresan seed treatment on stands and yields of 6 varieties of flax. Iowa, 1930*

Variety	Replications	Stand: Plants per 17′ Nontr.	Stand: Plants per 17′ Tr.	Stand: Tr. in per cent of nontr.	Acre yield: Nontr.	Acre yield: Tr.	Bushels increase	Tr. in per cent of Nontr.
Bison	26	.....	.....	.....	14.26	18.04	3.78	126.5
Bison	12	283.2	461.7	163.0	15.02	19.96	4.94	132.9
NDR-114	2	598	808	135.2	7.75	9.75	2.00	125.8
Redwing	2	456	671	114.7	11.75	13.25	1.50	112.6
Linota	2	359	819	228.0	7.00	9.00	2.00	128.5
Buda	2	252	445	176.4	4.50	6.50	2.00	144.5
Bison	2	223	331	148.1	6.50	9.00	2.50	138.5
Rio	2	124	247	199.0	6.75	8.25	1.50	122.1
Mean of six varieties	(12)	335.3	553.5	165.1	7.375	9.291	1.917	126.0

Table 6 shows that the stand and yield of every variety used were improved and that the mean increase for all the varieties was 65 per cent in stand and 26 per cent in yield.

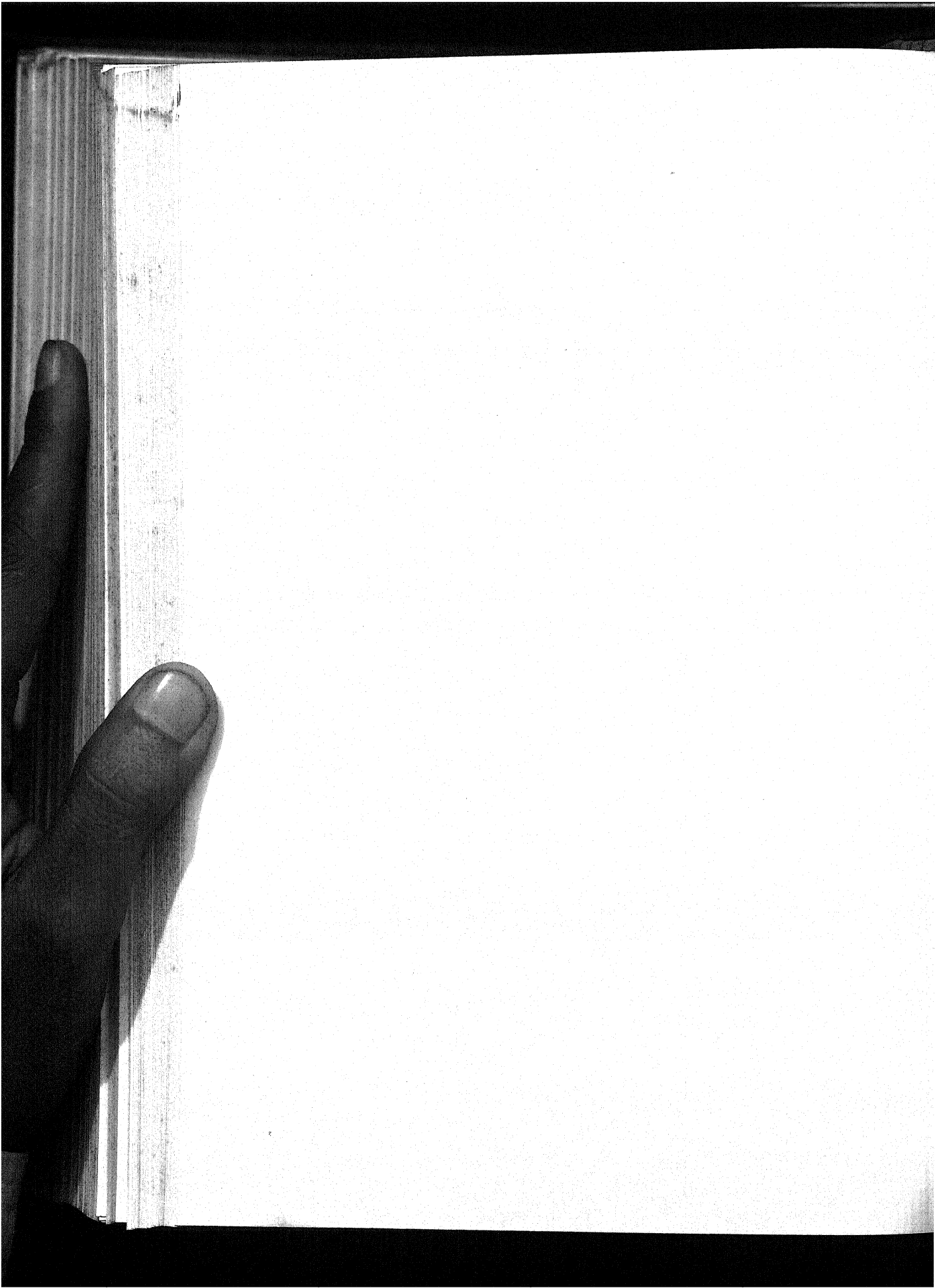
SUMMARY

Due to the exceptionally dry season, satisfactory yields of flax were obtained only from the first sowing, May 1; little more than half as much (53.4 per cent) from the second sowing, May 14; and practically no yield from the third sowing, May 31.

When Bison flax of high vitality was grown under a wide range of conditions the percentage of seed which produced plants varied from 14.9 to 53.4. In the same experiments seed of the same lot treated with Ceresan gave percentage stands of 42.6 to 63.6.

Seed treatment with Ceresan increased the yields of Bison flax 26.5 per cent in 26 replications and the yields of 5 other varieties (2 replications each) by percentages varying from 12.6 to 44.5.

IOWA AGRICULTURAL EXPERIMENT STATION,
AMES, IOWA.



PYTHIUM BUTLERI—THE CAUSE OF A BEAN WILT

L. L. HARTER AND W. J. ZAUMEYER[1]

INTRODUCTION

In a recent publication Harter and Zaumeyer[2] reported a wilt of beans, *Phaseolus vulgaris,* that occurred almost simultaneously in 1930 at Greeley, Colo., and Rosslyn, Va.

Not many days after the disease was observed in Colorado, one of the local growers reported that stem girdle, caused by *Bacterium phaseoli* E.F.S. or *Bact. medicaginis* var. *phaseolicola* Burk., was present in a number of bean fields and was causing the death of many plants. In examining these fields no bacterial blight was found. The death of the plants in this instance was caused by a Pythium which produced symptoms similar to those observed in other fields in the same general locality.

Pythium has long been known to cause bean-root rots and damping off of seedlings. However, this is the first time the writers have observed this organism to cause serious losses of almost mature plants under field conditions.

The purpose of this paper is to supplement the previous report of the disease and to give a more detailed account of the malady as it appeared under field conditions, with special reference to symptoms and conditions that favor its spread and development.

DESCRIPTION OF THE DISEASE

Pythium wilt begins as a water-soaked infection of the stem of the plant at about the soil line and progresses upward into the branches and petioles of the lower leaves. If the soil is drawn up to the stem, the initial infection is in the region of the lower branches. The organism progresses rapidly from the point of infection up the stem and into the branches, causing a water-soaked appearance of the surface. While there is no discoloration of the invaded tissue, the cortex is softened so that it can be readily separated from the vascular tissue, a characteristic said to be common to the Pythiums as a group. The infection rarely extends below the surface of the soil or to the roots.

The earliest symptoms of the disease are characterized by a slight wilting of the foliage in the warmer part of the day, followed by a return to the normal turgidity of the leaves at night. A few days later these symptoms are followed by pronounced continuous wilting in which the

[1] Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

[2] Harter, L. L., and W. J. Zaumeyer. A wilt of beans caused by Pythium. (Abst.) Phytopath. 21: 115. 1931.

branches droop noticeably, followed a few days later by death of the plant. If weather conditions are favorable, the mycelium will grow profusely over the dead cortex, often from the soil line into the branches. Under suitable conditions of temperature and humidity, the disease progresses so rapidly that the plant may be killed in a few days after infection.

HISTORY AND GEOGRAPHIC DISTRIBUTION

It is not believed that this disease has been observed previously on bean plants except as a damping off of seedlings and as a root rot. The distribution was not general in Colorado in 1930, but the disease was noted in one particular section where it was found in five large plantings, in some cases causing a reduction in stand of from 10 to 12 per cent. In most fields the amount was less. It was observed at Rosslyn, Va., for the first time in 1930.

SUSCEPTIBLE VARIETIES

It is believed that all snap-bean varieties are susceptible to the disease; however, it was observed under field conditions only on the Late Stringless Green Refugee, Round Pod Kidney Wax, Improved Kidney Wax, Hodson Wax, Giant Stringless Green Pod, and Black Valentine.

CONDITIONS FAVORABLE FOR INFECTION AND GROWTH OF THE FUNGUS

Pythium wilt of beans was observed in Colorado and at Rosslyn, Va., about July 15, 1930. In some respects the disease appeared under quite different climatic conditions and in other respects under similar ones. In Virginia no rain had fallen for several weeks. The soil, a light sandy loam, in which the beans were growing, was very dry and a dust mulch 3 or 4 inches deep covered the surface. The temperatures were extremely high when the disease was at its worst, ranging in the daytime from 32° C. to 41° C.

In Colorado the disease was observed on somewhat heavier soil. The water there is supplied mostly by surface irrigation, although rains may occur during the summer months. However, up to the time when the disease was noted, only 0.15 of an inch of rain had fallen. The season was very dry and the beans, which were from 10 to 12 inches high and growing vigorously, had been irrigated at least twice. The disease was observed in several fields close to one another and it was especially severe in a portion of one field that had been flooded by irrigation water. The results from this field alone would indicate that there is a direct correlation between wet soil and Pythium wilt. On the other hand, the disease occurred, with identical symptoms, in other fields nearby that had not been flooded with irrigation water.

Since the temperature was high in both Colorado and Virginia, the experimental data and field observations indicate that it was more likely to be the determining factor in the severity of the disease than soil moisture. It is generally believed that high relative humidity, and high moisture content of the soil are essential to the incidence of the diseases caused by Pythium.

Notwithstanding the fact that a drought prevailed in Virginia when wilt was prevalent, records taken by a hygrothermograph showed that the relative humidity during the night was close to saturation, a condition which would be favorable to infection by Pythium and its subsequent development. In irrigated fields of the arid regions of the West, a high relative humidity or nearly saturated atmosphere might and probably did occur at the crown of plants for several hours at a time. As a matter of fact, hygrothermograph records taken in irrigated bean fields of the West showed that, as in the East, the relative humidity during certain nights was quite high, although it did not extend over so long a period of time. The conclusion to be drawn from these data is that the relative humidity is sufficiently high for a long enough period of time in both the East and West to permit infection.

Pythium butleri, the cause of wilt of beans, is widely distributed. It occurs on the roots of many crops and is the cause of decay of beans in shipment. Harter and Whitney[3] showed that *P. aphanidermatum* causes a nesting and decay of beans in transit from the field in Florida to the northern markets. It has also been found on the fibrous roots of beans, and to be a cause of damping off of seedlings. *P. aphanidermatum* is now found to be *P. butleri,* the form that occurs on the roots of beans, and is the cause of nesting.

INOCULATION EXPERIMENTS

Cultures were made from diseased plants from both Colorado and Virginia, and the organisms from the two localities were identical. Inoculation experiments were made on seedlings and young plants beyond the seedling stage, by inserting mycelium into wounds made at the cotyledonary node. Some of the plants were covered with bell jars or inclosed in infection chambers to maintain a high humidity, while others were left uncovered. The percentage of infection was sufficiently high to demonstrate the causal relation of the fungus and reisolation proved the identity of the organism. The symptoms were typical of the disease as observed in the field under natural conditions. The checks remained healthy. Wilting of inoculated plants did not occur at ordinary greenhouse temperature, which

[3] Harter, L. L., and Whitney, W. A. A transit disease of snap beans caused by *Pythium aphanidermatum.* Jour. Agr. Res. 34: 443–447. 1927.

was maintained at about 21° C. during the daytime and about 16° C. at night. When these plants were placed in an infection chamber at 30° C. and in an almost saturated atmosphere, wilting occurred in three to four days.

It is believed that temperature is a more important factor than humidity in the occurrence of Pythium wilt. Evidence to that effect was obtained from a greenhouse experiment in which two sets of plants, each consisting of nine plants, were similarly inoculated by inserting the fungus into the stem at the soil level. Sphagnum was placed around the plants at the points of inoculation and kept moist in the one series, while the other series remained uncovered. Both sets, including the checks, were held at ordinary greenhouse temperatures and no infection resulted. When similar plants were placed in infection chambers at a temperature of 36° C., wilting followed in three to four days. It appears that a combination of high temperature and humidity provides ideal conditions for infection but that high temperatures are more essential for infection than high humidities.

REPORT OF THE FIFTEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY.

OFFICERS:

President.....E. Carsner, U. S. Department of Agriculture, Riverside, California.
Vice-President.....J. M. Raeder, University of Idaho, Moscow, Idaho.
Secretary-Treasurer.....B. A. Rudolph, U. C. Deciduous Fruit Station, San Jose, California.
Councilor.....C. E. Owens, State College, Corvallis, Oregon.

The meetings of the Pacific Division of the American Phytopathological Society were held in conjunction with those of the first national summer meetings of the American Association for the Advancement of Science and Affiliated Societies, at the California Institute of Technology at Pasadena, California, on June 16, 1931.

Thirty-five members, with friends and visitors, were in attendance. Twelve papers were read, the titles and abstracts of which are included in the present report.

The meetings this year were designed to permit of a great number of interesting excursions, many of which were enjoyed by visiting members. On Monday, June 15, the members of the American Association for the Advancement of Science and Affiliated Societies were accorded a reception and private showing of the magnificent Huntington Gardens at San Marino, a suburb of Pasadena. On this same occasion the famous library and art gallery also were thrown open to members of the Association. An orchestra played out of doors under the great oaks, which are one of the fine features of the gardens, and refreshments were served throughout the afternoon.

On Friday, June 19, the Pacific Division pathologists met with Section G of the American Association for the Advancement of Science and Affiliated Societies at the Rancho Santa Ana Botanic Gardens as the guests of Mrs. Susanna Bixby Bryant, its founder. Papers pertinent to botanic gardens were read by Douglas H. Campbell, Walter T. Swingle, and H. J. Webber. Following a delicious luncheon a visit was made to the botanic gardens on the estate.

Many other free and interesting excursions to the beaches, moving-picture studios, the Mount Wilson Observatory, etc., were made possible through the courtesy of the Los Angeles Chamber of Commerce and other organizations.

B. A. RUDOLPH, *Secretary-Treasurer*

ABSTRACTS

The curing of exanthema by the injection of copper sulphate into the tree.—H. E. THOMAS.

Results obtained in central California over a period of 2½ years indicate that powdered copper sulphate, injected during the dormant season into holes bored in the base of the tree trunk, may successfully control exanthema in deciduous fruit trees. The amount necessary to cure the disease appears to vary. Two grams was insufficient at times to eliminate all burning in large trees. Injections of 15 and 20 gm. did not cause injury. While the addition of copper sulphate to the soil around the base of diseased trees in one orchard failed to decrease noticeably the amount of burning in the top, the injection of from 4 to 6 gm. of the sulphate into the crown produced trees entirely free of burn or dying tips. Limited trials indicate copper acetate and chloride effectively cure the disease. Copper tartrate, phosphate, and carbonate failed to give satisfactory results.

Speculation arises as to the rôle of copper in this disease. It may be necessary as an essential element for the growth of the tree, diseased trees having insufficient copper; or it may act in neutralizing some toxic substance taken up by the tree from the soil in which the tree is growing.

Developmental stages of Heterodera radicicola in pineapple and cowpea roots.—G. H. GODFREY and JULIETTE OLIVEIRA.

Large numbers of young, actively growing roots of pineapple and cowpea were heavily inoculated with recently hatched larvae of the root-knot nematode and then, at regular intervals, roots were excised, washed, killed in Fleming's, fixed, dehydrated in graded alcohols, and cleared in clove oil. The results were series of roots showing clearly the successive stages of nematode development and relationship to the root tissues, beginning with earliest penetration and ending with the initiation of a new generation. The nematodes were stained dark and stood out clearly in their natural position in the root tissues. A series of photographs of about 16-diameter magnification records this graphically. Permanent slides were made by infiltration with balsam. A striking development from this study is that the length of life history varies greatly between the two host plants, growing conditions being identical, as both plants were inoculated at precisely the same time. The life cycle is much longer in the pineapple roots.

Verticilliosis of strawberries.—H. E. THOMAS.

It appears that *Verticillium albo-atrum* is capable of causing a disease in strawberries. Plants set at San Jose, California, in soil accidentally infected with Verticillium were showing marked symptoms of distress 3 months after planting. The outermost leaves of infected plants wilt, turn brown, and die, or burn at the edge, leaving an irregular green center in the leaf. Affected plants may die slowly but often partially recover. Occasionally, a rather sudden wilt occurs. Verticillium was readily isolated from the crowns of such plants but was never taken from the roots. Several dying plants taken from commercial patches yielded the fungus upon culturing from the crowns. Thirty strawberry plants of the Nick Ohmer variety were set in sterilized soil in 6-inch pots in the greenhouse and inoculated with pure cultures of *Verticillium albo-atrum*. Five months later 50 per cent of the inoculated plants showed wilting and dying in varying degree, similar to that occurring in the field. The remaining 50 per cent appeared to be unaffected by the inoculation. Twenty check plants remained healthy. The fungus was isolated from the crowns of the diseased plants but not from the checks.

A new disease of maize and beans.—W. W. MACKIE.

In the summer of 1929 a new disease was found in maize and beans. The fungus was identified as *Rhizoctonia bataticola* (Taub.) Butler. It is easily recognized by the presence in the stele of dense black sclerotia about 120 to 200 microns in diameter. It is transmitted from sclerotia to sclerotia in maize and beans. In culture media about 6 days are required to again reproduce the sclerotia. In beans and maize the sclerotia are found in the roots and in the plant stems to a point not beyond 10 or 12 inches above the soil. In beans the black sclerotia are found deeply embedded in the stele. In maize and beans they cluster about the vascular bundles but are found also in the pith. Wilt and premature ripening ensue, with a reduction in the size of the plant and the seed. All commercially grown beans in California, except Large Limas, and all subspecies of maize were attacked. Resistant varieties were found.

A preliminary report on resistance to curly top of sugar beets in bean hybrids and varieties.—W. W. MACKIE and KATHERINE ESAU.

Since 1919 curly top of sugar beets has been known to attack beans. To test the resistance of bean hybrids and varieties short rows were infested with infective nymphs of *Eutettix tenellus*, at the fourth-leaf stage, with the following results.

Grades of resistance	1		2		3		4		5	
	Immune, or no visible damage		Slight injury		Considerable injury		Heavy injury		Destruction	
	No. of varieties	Per cent	No. of varieties	Per cent	No. of varieties	Per cent	No. of varieties	Per cent	No. of varieties	Per cent
Phaseolus vulgaris										
White	3	4.7	3	4.7	9	14.0	16	25.0	33	51.6
Pink or rose	5	35.6	2	14.2	2	14.2	1	7.0	4	29.0
Mottled	2	20.0	3	30.0	1	10.0	1	10.0	3	30.0
P. multiflorus										
White			1	100.0						
P. lunatus sieva										
White	0	0.0	1	17.0	4	66.0	1	17.0		

In the cross between Robust, a white pea bean, resistant to mosaic, and California Pink, resistant to curly top, a number of fixed resistant hybrids were secured in both pink and white beans. Correlation between pink or red color and resistance was found to be only partial, indicating that white beans can be secured that are resistant to curly top. Continued resistance to both curly top and bean mosaic may be secured in both white and pink beans of the species *Phaseolus vulgaris*.

Experimental freezing of apple trees.—J. H. CRENSHAW and J. S. COOLEY.

In experimental studies of perennial apple canker it seemed desirable to freeze parts of apple trees having received different preparatory treatments.

Method: The limb to be frozen was fitted into notches in the top of an insulated box containing the refrigerant. Two different refrigerants were used. A mixture of $CaCl_2$ and snow gave a minimum temperature of $-25°$ to $-35°$ F., but required recharging at 3- to 5-hour intervals. By using solid CO_2 in definite amounts as the refrigerant, temperatures could be controlled as low as needed by recharging at intervals of 24 to 36 hours. A temperature of $-40°$ F. was easily obtained. Freezing was done across a fertilizer plot at three different times, viz., late fall, midwinter, and early spring. The types of wounds frozen were healthy calluses, old cankers, and fresh pruning wounds. Some of the calluses had been kept free from aphis, while others were aphis infested.

Results: The killing resulting from freezing on November 30 was much severer than that on January 1. Callus tissue was more susceptible to freezing than normal bark. Woolly-aphis-infested calluses were more susceptible to cold injury than aphis-free

calluses. Fresh wounds were more severely injured than callused wounds. Cankers were more susceptible to injury than sound-healing calluses. No positive correlation between fertilizer treatment and freezing injury was noted.

Tranzschelia punctata on cultivated anemone in the Santa Clara Valley.—C. EMLEN SCOTT and GILBERT L. STOUT.

In March, 1931, attention was called to an anemone planting near San Jose in which a rust infection was severe. Leaves of almond, apricot, cherry, nectarine, peach, plum, and prune, in Petri dishes, were inoculated by dusting with aeciospores from these anemones. Infection took place and uredinia containing urediniospores typical of *Tranzschelia punctata* were produced in 12 to 20 days on all of the hosts except cherry. The aecial stage of the stone-fruit rust has not been reported previously from the Pacific Coast, but specimens of cultivated anemones bearing aecia, collected at Salem, Oregon, in 1926, and received from H. P. Barss, as well as similar material collected near San Jose, California, in 1927, by L. R. Cody, are deposited in the pathological herbarium of the California Department of Agriculture.

Black scorch of the date palm.—L. J. KLOTZ and H. S. FAWCETT.

A fungus disease of economic importance has been found on date palms in California, Arizona, and Northern Africa. Preliminary survey indicates that all varieties of the date palm probably are susceptible. The disease has been found occurring naturally on all parts of the plant except the roots and stem, and these latter organs have, by artificial inoculation, been found readily susceptible. The most typical lesion is a dark brown to black, hard carbonaceous, scorching effect on petioles, midrib, and fruit strands and fruit stalks, which suggests *black scorch* as the common name. Many of the fruit strands may be completely severed by the attack and the crop materially lessened. Wounding was shown to be unnecessary for infection of fruit strands. The decay is most serious where it attacks the terminal bud, either killing the palm or, when not fatal, producing the so-called "fool-disease" effect, in which the injured terminal bud grows out laterally, setting the normal growth of the palm back several years. Both the hyaline and the brown spores of the fungus *Thielaviopsis* sp., probably *T. paradoxa* (De Seynes) von Höhnel, are found on the surface of the lesions. The conidia originate endogenously in uniseriate chains from subhyaline conidiophores. The optimum temperature for fungus in culture lies between 24° C. and 27½° C.; it makes very little growth at 32° C. The brown spores apparently need a rest period before germination. The hyaline conidia germinate readily without a rest period, sending out one, occasionally two, germ tubes from any place on its periphery. In germinating, the protoplast of the mature macroconidium bursts through a longitudinal slit liberating a globule of naked protoplasm which proceeds to grow into mycelium. The hyphae are subhyaline with cross walls and show a strong tendency to anastomose and form branches at right angles to the parent hyphae.

To control the malady, the affected fronds, leaf bases, and inflorescences should be pruned out and the pruning cuts and surrounding tissues disinfected. Preliminary experiments indicate that copper sprays and dusts may be effective.

Graft transmissions of curly top in tomatoes (tomato yellows).—M. SHAPOVALOV.

Young, healthy tomato plants, about 3–4 inches high, grown in pots under greenhouse conditions at Berkeley, were inoculated by exposing one leaf of each plant to

viruliferous beet leaf hoppers, *Eutettix tenellus* Baker, for 1 week. The inoculated plants were then grafted by the approach method with healthy plants of the same age. Some of these plants were shaded. On the average a higher number of transmissions was obtained by inoculation with viruliferous insects than by grafting. The percentage of graft-transmitted cases of the disease was nearly equal to that of the insect-transmitted cases when grafts were made immediately after the removal of the insects. When there was an interval of 2 or more days between these two operations, a certain percentage of the grafts failed to show symptoms of the disease. Some of the plants that did not show the symptoms also failed to show the presence of the virus when tested with nonviruliferous insects fed afterwards to healthy sugar beets. Exposure of inoculated and grafted plants to additional artificial light in the evening, produced by four 500-watt Mazda lamps, for the duration of 4 to 6 hours, accelerated the rate of disease development, whereas muslin shading retarded it. No significant changes appeared in the final percentages of the disease.

Streak, a virus disease of peas transmitted by Thrips tabaci.—M. B. LINFORD.

A disease of canners' peas, *Pisum sativum* L., characterized by a streaked and spotted brown necrosis of pods, stems, and leaves, was found by the writer, in 1928, widely distributed across the United States. On pods necrotic circular pitting may develop, or the whole pod may collapse. On leaves the injury may begin with spotting or with brown vein streaking extending down the stems as a phloem necrosis. The pods alone, the stem apex, or the entire plant may become necrotic. No microorganisms have been found associated with this disease. During investigations of pineapple yellow spot in Hawaii, infective thrips, *Thrips tabaci* Lindeman, were transferred to peas from infected *Emilia sagittata* (Vahl) DC. Symptoms developed that appear identical with pea streak and that do not follow feeding of noninfective thrips. Of 45 plants so treated, 21 have become infected. Individual thrips have transmitted infection. Thrips reared on infected peas have transmitted to peas, reproducing streak, and to pineapple, producing typical yellow spot. In peas the incubation period is about 12 to 20 days. Streak caused by the yellow-spot virus occurs in market-garden plantings near Honolulu. It is suggested that streak on the mainland is caused by either this or a related virus.

Further studies of transmission of the pineapple yellow-spot virus by Thrips tabaci.—M. B. LINFORD.

Further studies of the transmission of the pineapple yellow-spot virus by *Thrips tabaci* Lindeman have revealed a specialized relationship between virus and insect. *Emilia sagittata* (*E. flammea*) was the chief test plant, supplemented by pineapple and others. The thrips were from three noninfective colonies reared each from an individual larva. The yellow-spot virus is transmitted by both adults and large larvae reared on infected plants. The virus survives pupation. A single insect may transmit. When adults from noninfective colonies are allowed to feed upon diseased plants they appear never to become infective. Progeny of these adults, allowed to develop on the same diseased plants, do become infective. Also, larvae from noninfective colonies allowed to feed upon diseased plants become infective and may transmit the virus while either larvae or adults. The failure of adults to become infective has been shown consistently in tests with thrips from a uniform source exposed to four species of host plants as the source of virus. In the larvae there is an incubation period lasting approximately 10 days. This virus is not readily transmitted by mechanical means. Host plants of the yellow-spot virus include members of several unrelated families.

Relation of perennial apple canker to its environment.—JACQUELIN S. COOLEY and ELMER V. SHEAR.

Perennial apple canker has a very restricted geographical range, it being confined largely to the valleys of the Cascade Mountains. A study was made of the yearly and seasonal rainfall and minimum winter temperature in fruit regions of the Pacific Northwest where perennial canker is present or absent. There appears to be a definite correlation between the severity of canker infection and winter precipitation only in areas where temperature is sufficiently low. Results are given confirming last year's report that local callus winter injury serves as the main court of infection for the canker fungus. Experimental infections, however, have been obtained without winter injury. Evidence shows that woolly aphis infestation may increase the susceptibility of calluses to winter injury, with resulting canker growth. Both natural and experimentally produced callus, winter injury, and subsequent canker infection have been recorded where there had been no aphis infestation. In the fall of 1930, at the higher elevations in the Hood River Valley, where fall rains and spore discharge occurred before fruit harvest, canker rot of fruit in storage was severe. In the Lower Valley spore production occurred later, due to temperature conditions and the absence of rain. Fruit harvest was earlier, with the result that there was little loss from canker rot in storage.

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AN EXPERIMENTAL INVESTIGATION OF SEX IN THE RUST FUNGI[1]

J. H. CRAIGIE

INTRODUCTION

The rusts comprise a distinct group of the Basidiomycetes. They are all parasitic, and some of them may, under favorable conditions, cause destructive epidemics. On account of their obligate parasitism, they present considerable difficulties to cultural studies. Owing to this fact, possibly, certain aspects of their developmental cycle have remained obscure. They have, however, been the subjects of a great deal of investigation. The black stem rust of cereals has been studied more than any other rust fungus. In general, it may be said that the amount of study given to a particular species has been about proportional to its economic importance.

This paper is a contribution to our knowledge of the rust fungi, particularly in respect to their sexuality; and the observations about to be recorded have been made on *Puccinia graminis* Pers., *P. helianthi* Schw., *P. coronata* Corda, *P. Pringsheimiana* Kleb., and a species of Gymnosporangium.

HISTORICAL SUMMARY

Our knowledge of the life history of rust fungi made a great advance in the middle of the nineteenth century. Previous to that time each spore form was regarded as belonging to a distinct genus. Tulasne (79), in 1854, showed that the uredinial and telial sori that are found on stems of wheat are not derived from two rust species, as had previously been believed, but that they are products of the mycelium of one and the same species, namely, *Puccinia graminis*. In 1865, de Bary (17) established experimentally the genetic connection of the two stages of the rust fungus on wheat with the aecial stage (*Aecidium berberis* Pers.) on the barberry. He inoculated barberry plants with sporidia from germinating teliospores. From these inoculations arose pycnia and aecia. Wheat plants inoculated with the aeciospores produced uredinia and telia. Thus he demonstrated

[1] Contribution from the Division of Botany, Department of Agriculture, Ottawa, Canada. This memoir was awarded the Eriksson Prize for Cereal Rust Investigations, 1930. It was submitted to the University of Manitoba in the spring of 1930 as a thesis in partial fulfilment of the requirements for the Ph.D. degree.

that *P. graminis* produces five spore forms which appear in regular sequence: pycniospores in pycnia; aeciospores in aecia; urediniospores in uredinia; teliospores in telia; and sporidia on the basidium derived from a germinating teliospore.

Pycnia were discovered by Unger (81) in 1833 and were considered by him to be the fructification of a distinct fungus. Since that time the pycnia have been the object of much speculation. Meyen (50) noticed the close association of pycnia and aecia and suggested that they represented the sexual organs of one and the same fungus. This view was supported by Tulasne (80). As the pycniospores were apparently lacking in power of germination and as the pycnia bear a striking resemblance to structures in collemaceous lichens, believed to be sexual in nature, Tulasne (78) called the pycnia spermogonia. De Bary (16) inclined to the view that the spermogonia of the rust fungi are male organs, producing spermatia which are apparently incapable of germination; but he pointed out that pycnia (spermogonia) are unaccompanied by aecia in some rusts, and, in some other rusts, aecia are unaccompanied by pycnia. He therefore considered it best, until further knowledge became available, to regard the pycnia as organs whose physiological significance was doubtful. The germination of pycniospores of rust fungi was observed by Cornu and Roze (11), and the germination of the spermatia of lichens by Möller (51). They regarded these spore forms as conidia. The fact that pycniospores had been observed to germinate and that aecia are sometimes unaccompanied by pycnia led Brefeld (8) to oppose the idea of sexuality in the rusts and to consider the pycnia not as spermogonia but as asexual reproductive organs to which the name pycnidia should be applied. Klebahn (33) opposed the view that the pycnia are male organs but pointed out that the pycniospores do not behave like conidia. Plowright (61) arrived at much the same conclusion. If the pycniospores are conidia, they should, in his opinion, be able to infect the host plant, but he was never able to prove such infections experimentally. However, he observed the germination of pycniospores in sugar solutions.

The opinion of Brefeld that the pycnia are not male organs was more or less generally accepted until 1904, when Blackman (6) revived the theory of the sexual nature of the pycnia. He supposed that the pycniospores were once functional, like the spermatia of the red seaweeds, but that they became functionless when the trichogynes of the rusts disappeared. In support of his view that pycniospores are functionless male cells, he referred to the density of their nuclei, the paucity of their cytoplasm, and the thinness of their cell wall. In addition, he pointed out that pycniospores possess but feeble power of vegetative development and are (as far as known) incapable of producing infection. Christman (10) in-

clined to the view that the pycnia once produced functional asexual gametophytic spores—conidia—as do the pycnidia of Ascomycetes. He questioned the belief that the pycniospores are functionless, although he admitted that their nature was not very evident. It was McAlpine's (47) belief that the pycnia are isolated organs and that, whatever their original function may have been, they are now quite functionless. Grove (25) advanced eight reasons in support of the assumption that the pycniospores represent functionless male cells. Later investigators accepted one or other of these two theories, but the opinion was fairly unanimous that the pycnia, whether they were originally asexual gametophytic reproductive organs or sexual organs producing spermatia, are no longer functional. The view that the pycniospores are functionless was very definitely expressed by Gwynne-Vaughan and Barnes (26) in their text-book published in 1927.

Cytological investigations have made important contributions to our knowledge of the rust fungi. In 1880, Schmitz (67) discovered paired nuclei in the mycelium and urediniospores of *Coleosporium campanulae* (Pers.) Lév. Two nuclei were seen by Rosen (64) in the aeciospores of *Uromyces pisi* (Pers.) de Bary and in the young teliospores of *Puccinia asarina* Kunze but only one in the mature teliospores. Poirault and Raciborski (62) introduced the term "conjugate nuclei" for the paired nuclei and made some observations on the chromosomal behavior during nuclear fusion and reduction. A comprehensive study of the mycelia and fructifications of a large number of rust species was made by Sappin-Trouffy (66). He found that the pycniospores and the hyphae which produce them are uninucleate; that the aeciospores, urediniospores, and the hyphae arising from them are binucleate, as are also the cells of the immature teliospores, but that, before germination, the cells of the teliospores become uninucleate through the fusion of their conjugate nuclei. Two divisions occur in the basidium, one of which is a reduction division, so that the four basidiospores are uninucleate and produce uninucleate mycelia. He considered that the fusion of the two nuclei in the teliospore represents a true sexual process but paid little attention to the origin of the binucleate condition in the aeciospores.

The origin of the binucleate condition was investigated by Blackman (6), who found that in *Phragmidium violaceum* Wint. the nucleus of one cell migrates through a pore into a neighboring cell. He interpreted this as the beginning of a sexual act which culminates in the fusion of the two nuclei in the teliospore. Christman (10), in a similar study on *P. speciosum* Burrill, found that fusion occurs between two neighboring cells and suggested that the nuclear migration observed by Blackman might have been due to a pathological condition. Nuclear migration also has been observed by Welsford (89), Kursanov (38), and several others; but

most of the later workers, Olive (58), Kursanov (37), Fromme (23), Maire (46), Lindfors (42), etc., have found that the conjugate condition of nuclei in the spore bed of the aecia of many rust species is due to cell fusion, although both types have been found in some species (38, 42, 58). Very recently, Hanna (29) has shown that cell fusion occurs in *Puccinia graminis*.

The phenomenon of heterothallism is known to occur in the Phycomycetes, the Ascomycetes, and the Basidiomycetes. It has been demonstrated experimentally: in the Phycomycetes, by Blakeslee (7) in Mucor and other Mucorineae, and by Couch (12) in Dictyuchus; in the Ascomycetes, by Dodge (20) in *Ascobolus magnificus* Dodge, by Betts (5) in *A. carbonarius* Karst., by Derx (18) in *Penicillium*, by Wieben (90) in *Taphrina*, and by Shear and Dodge (69) in *Neurospora sitophila* Shear & Dodge and *N. crassa* Shear & Dodge. Dodge (21) secured fertile hybrids from a cross between *N. sitophila* and *N. tetrasperma* Shear & Dodge.

In the Hymenomycetes heterothallism has been shown to occur: by Mlle. Bensaude (4) in *Coprinus fimetarius* (Lin.) Fr.; by Kniep (34, 36) in *Schizophyllum commune* Fr. and *Aleurodiscus polygonius* (Pers.) v. Hoehn. & Litsch; by Miss Mounce (53, 54) in *Coprinus lagopus* Fr., *C. niveus* Fr., and *Fomes pinicola* Swartz; by Vandendries (82, 83, 84) in *Collybia velutipes* Court., *Hypholoma fasiculare* Huds., *Panaeolus campanulatus* L., *P. separatus* L., *P. fimicola* Fr., and *Coprinus radians* Desm.; by Brunswik (9) in a number of species of Coprinus; by Miss D. E. Newton (55) in *Coprinus Rostrupianus* Hansen; and by Miss Gilmore (24) in *Psilocybe coprophila* Bull.

Among the smut fungi, Kniep (35) found evidence of heterothallism in *Ustilago violacea* Pers.; Stakman and Christensen (71), in *U. zeae* (Beckm.) Ung.; Dickinson (19) in *U. levis* (Kell. & Sw.) Magn. and *U. hordei* (Pers.) Kell. & Sw.; and Hanna (28) in *U. zeae* and *Sorosporium reilianum* (Kühn) McAlp.

Until 1894, *Puccinia graminis* was considered to be a single species capable of attacking all of the common cereals and grasses, but in that year Eriksson (22) showed that it consisted of several pathogenic strains or forms, each of which is specific for certain gramineous hosts. These strains he designated as "formae speciales" and classified them according to their infective capabilities as follows: *P. graminis tritici* Eriks. & Henn. on wheat, *P. graminis avenae* Eriks & Henn. on oats, *P. graminis secalis* on rye, *P. graminis airae* Eriks. & Henn. on Aira, *P. graminis agrostis* Eriks. & Henn. on Agrostis, and *P. graminis poae* Eriks. & Henn. on Poa. All had the common barberry as an aecial host. Results of a confirmatory nature were obtained by Rostrup (65), Magnus (43), Hitchcock and Carleton (30), Klebahn (32), and Ward (87).

A careful study by Stakman and Piemeisel (76) in 1917 revealed that *Puccinia graminis tritici* was not a simple form but consisted of at least 2 pathogenic strains. In the following year, a third strain was identified by Melchers and Parker (49) and a fourth one by Levine and Stakman (40). By using twelve standard varieties of wheat, called "differential hosts," Stakman and his coworkers (72, 75, 77) have been able to distinguish a large number of pathogenic forms by their reaction on these hosts. Newton, Johnson and Brown (56) have reported 8 additional forms. As the morphological characters of all these strains are more or less identical, the dissimilarity of their reactions is considered to be due to physiological differences in the strains, and, hence, the strains are now referred to as "physiologic forms."

Not only has physiologic specialization been found in *Puccinia graminis tritici,* but it has been demonstrated by Stakman, Levine, and Bailey (73) in *P. graminis avenae* and by Levine and Stakman (41) in *P. graminis secalis.* It has also been shown to occur in other rusts. Mains and Jackson (45) distinguished 12 forms in *P. triticina* Eriks.; Mains (44), 4 forms in *P. sorgi* Schw. and 2 in *P. anomala* Rostr.; Hoerner (31), 4 forms in *P. coronata* Corda; and Bailey (3), at least 3 forms in *P. helianthi* Schw. These citations are sufficient to indicate that the phenomenon of physiological specialization occurs more or less generally among the rust fungi.

From the historical summary just concluded, it is evident that prior to 1926, at which time the investigation here recorded was begun, many fundamental facts concerning the life history of *Puccinia graminis* had been brought to light; it was known that this rust is heteroecious; that fusion of the conjugate nuclei occurs in the teliospores prior to germination; that reduction takes place in the basidium; that the sporidia give rise to uninucleate mycelia; and that the aeciospores are binucleate. By inference from what was known to occur in other rusts, the binucleate condition of the aeciospores was supposed to arise either by cell fusion or nuclear migration in the aecial spore bed. It was known also that physiological specialization in *P. graminis* is very pronounced. And, finally, it was generally agreed that, whatever the original function of the pycnia may have been, that function had been lost.

No one up to the year 1927 ever had studied the problem of sex in *Puccinia graminis* or any other rust experimentally; but, in that year, the writer was able to announce that *P. graminis* is heterothallic and that the pycnia are functional (13, 14). A further communication from the writer (15) in 1928 contained additional observations on sex in the rust fungi.

PROBLEM STATED

Black stem rust (*Puccinia graminis*) is one of the most destructive diseases of cereal crops. As already stated, *P. graminis* is not a simple

species but comprises a large number of physiologic forms. The fact that one cereal variety may be highly resistant to one or more physiologic forms but quite susceptible to other forms has very materially impeded the plant breeder in his effort to produce rust-resistant varieties of cereals. Whether or not new physiologic forms arise from time to time under natural conditions was not known, but it was argued theoretically that if *P. graminis* is heterothallic any two physiologic forms of this organism may hybridize on the barberry and so produce new forms which may render breeding for rust resistance more or less futile. Stakman, Levine, and Leach (75), as early as 1919, mentioned the possibility of hybridization of physiologic forms occurring on the barberry. Whether or not hybridization does take place is a question of very practical importance, as well as one of considerable scientific interest. The writer undertook to investigate this problem.

It seemed obvious to him that the first step in the investigation was to discover whether *Puccinia graminis* is homothallic or heterothallic. Both homothallic and heterothallic species are known among the Basidiomycetes. No sexual differentiation was found by Miss Mounce (52) to exist in the basidiospores of *Coprinus sterquilinus* Fr. or of *C. stercorarius* Gillet, or by Brunswik (9), in the basidiospores of *C. narcoticus* Fr. or of *C. ephemeroides* Fr. On the other hand, Kniep (35) showed that the basidiospores of *Ustilago violacea* are divisible into two sexual groups, and a similar condition was demonstrated by Vandendries (84) in *C. radians* and by Miss D. E. Newton (55) in *C. Rostrupianus.* Four sexual groups of spores were found by Kniep (34, 36) in *Schizophyllum commune* and *Aleurodiscus polygonius,* and by Hanna (27) in *C. lagopus.* It was therefore possible that one of these three conditions might exist in *P. graminis.*

If the sporidia of this rust are not differentiated for sex, or, in other words, if the species is homothallic and its sporidia are all sexually alike, an infection of a barberry leaf by a single sporidium should produce a pustule in which normal aecia would arise. If the sporidia were differentiated for sex, or, in other words, if the species is heterothallic and its spores are divisible sexually into 2 or 4 groups, an infection by a single sporidium should produce a mycelium which would remain in the haploid condition. Aecia would not be expected to develop, therefore, in such a pustule, unless indeed, a spontaneous change from the haploid to the diploid condition occurs, similar to that found by Vandendries (85, 86) in *Coprinus radians* and *C. micaceus* (Bull) Fr., and by Miss D. E. Newton (55) in *C. Rostrupianus.* Aecia might be expected, however, to arise in a compound pustule formed by the coalescence of two monosporidial pustules of opposite sex, but not in a compound pustule formed by two coalescing pustules of the same sex or of two sexes incapable of interacting sexually.

In attempting to elucidate the sexual condition of *Puccinia graminis* from the point of view of the possibilities just discussed, it was necessary to obtain two kinds of pustules: (1) simple pustules, each derived from the sowing of a single sporidium and (2) compound pustules, each derived from the sowing of two sporidia very close to each other and from the eventual coalescence of the two simple pustules resulting therefrom.

MATERIALS

As the primary object of the work was a study of the sexual behavior of *Puccinia graminis,* this rust was used throughout the investigation, and exclusively in 1926. For reasons that will be mentioned later, *P. helianthi* was used extensively in the experiments of 1927. The source of the telial material of *P. graminis* was heavily infected culms of wild barley, *Hordeum jubatum* DC., and that of *P. helianthi,* withered leaves of the cultivated sunflower, *Helianthus annuus* L. Collections of both rusts were made at the Agricultural College, Winnipeg, Manitoba.

For convenience in handling, only small plants of the respective hosts were employed. The barberry plants varied from 6 to 12 in. in height and grew singly in 6-in. flower pots. Sunflower seedlings were grown in similar pots, usually 4 or 5 to a pot. They were inoculated as soon as the first foliage leaves were 1 in. in length.

Owing to the fact that barberry leaves become very highly resistant to infection after they are 12 days old, only young leaves of this plant were successfully inoculated. Moreover, as a leaf grows older, it appears to offer progressively greater resistance to the spread of the mycelium through its tissues. The older the leaf when infected, the smaller usually is the pustule that arises from the infection.

METHODS

Inoculations were made by two methods, A and B. By method A, sporidia were picked off their sterigmata and deposited, either singly or in pairs, in a drop of water on the leaf of the host plant. By method B, sporidia were sown sparsely over the leaves of the host plant.

Method A was employed exclusively during 1926. It was not, however, very productive of results, but as it was the method by which the first results were obtained and as, in the opinion of the writer, the comparative failure attending its employment was not inherent in the mechanism or manipulation of the spore-picking apparatus but attributable to another factor, a description of the apparatus and its operation will be given in detail.

(1) *Method A*

Before inoculations could be made by this method, it was necessary to construct a spore-picking device which was amenable to rather quick

manipulation. A drop of water begins to form at the base of a sporidium just about 15 seconds before the sporidium is discharged. The formation of this drop indicates that the sporidium is mature. Only mature sporidia were desired for inoculating barberry leaves. Contact had to be made with a sporidium while its drop was being excreted, *i.e.*, within a period of 15 seconds. Also, a moist chamber was required in which an optimum humidity for teliospore germination could be maintained and still permit ready access to an instrument to pick off the sporidia.

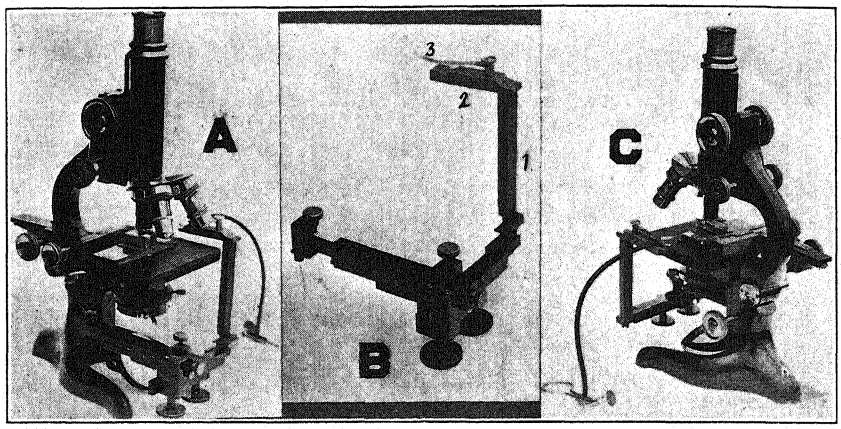


FIG. 1. A. View of right-hand side of microscope showing one mechanical stage in the usual position and another inverted and clamped to the projection on the substage which supported the iris diaphragm. B. View of mechanical stage in inverted position showing: the upright pillar, *1;* the horizontal arm, *2,* with its curved notch (towards the left end); and the spring clip, *3*. The stage is ready to be attached to the microscope. C. View of left-hand side of microscope showing capillary tube with attached rubber tubbing in position and ready for use.

Apparatus. The iris diaphragm was removed from a Leitz microscope and to the projection which supported the diaphragm was clamped in an inverted position a mechanical stage (Fig. 1, A). The swing arm of the stage was disjoined and at its place of attachment was erected a wooden pillar, 1, as shown in fig. 1, B. At the top of 1, a short wooden arm, 2, was fixed at right angles. On the upper side of 2, a spring clip, 3, was firmly secured.

A short piece of glass tubing, 5 mm. in diameter, was drawn out to a fine capillary bore. The unreduced part was fitted into the end of a piece of soft rubber tubing about 8 in. in length and of the same diameter as the glass tube. Thus connected, the two were mounted as seen in figure 1, C. The part of the glass tube covered by the rubber tubing was placed in the notch (Fig. 1, B) cut on the upper side of 2 and was held in position by

the spring clip, 3. The capillary part of the tube reached to the proximal edge of the field of view of the microscope; and the free end of the rubber tubing, closed by a spring clamp, rested on the table (Fig. 1, C).

It was then possible to raise or lower the capillary tube by means of the rack and pinion which served to adjust the substage of the microscope; and to move it from right to left, or from front to rear, within limits, by the inverted mechanical stage.

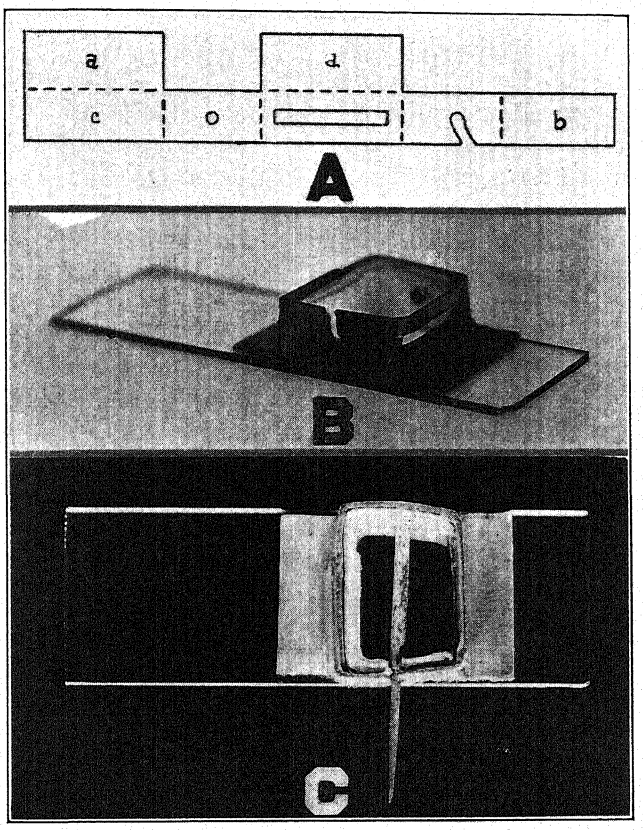


FIG. 2. A. Pattern to which a piece of Bristol board was cut to form the walls of a moist chamber. The Bristol board was folded along the dotted lines so that the two wings, marked *a*, projected outward and lay flat on the surface of the glass slide. The narrow end, marked *b*, overlapped the corresponding portion of the other end, marked *c*, and was glued to it. Slightly reduced. B. Moist chamber showing rectangular opening in front to the right, the notch and hole in the two side walls, and the filter-paper pad. Slightly reduced. C. Top view of moist chamber showing the filter-paper lining (white) and the piece of straw bearing telia mounted on the pin and ready for use. Actual size.

To form a moist chamber, a heavy strip of Bristol board was cut to the pattern shown in figure 2, A, and folded along the dotted lines; so that, when completed and cemented to a glass slide, it presented the appearance shown in figure 2, B. Before being cemented to the slide, it was dipped in liquid paraffin to prevent water absorption when in use. The rectangular opening in the front (Fig. 2, B) afforded access to the interior. A filter-paper pad lined the interior of the other three sides. Midway across the chamber lay a small wooden pin (Fig. 2, C), supported at one end by a small hole in one side wall of the chamber and, at the other end, by the notch cut in the opposite side wall. The pin projected about half an inch beyond the side of the chamber so that it could easily be reached by the hand and be rotated by the thumb and finger.

Preparation of inoculum. A straw bearing numerous telial sori was soaked in water for about 1 hour. From it was cut off a piece just long enough to reach across the moist chamber. The wooden pin was passed through the hollow center of the piece of straw and was then replaced in the chamber, as shown in figure 2, C. Sufficient water was added to the filter-paper pad to wet it thoroughly. Thus both ends of the piece of straw were in contact with water, and, so long as the filter-paper remained wet, the telial sori were kept damp. To prevent the mount from drying during the ensuing night, the moist chamber (all the apparatus shown in figure 2, C) was set in a Petri dish, the bottom of which was covered with water.

To secure basidia which projected sufficiently from the straw, it was necessary to keep the telial sori covered with a thin film of moisture. No exact data on the optimum moisture requirement were taken. Experience alone served as guide. But when a certain thickness of film was present, the basidia were somewhat longer and stood well out from the sori, in which case it was easy to bring the capillary tube into contact with individual sporidia. If too much moisture was present, the basidia were distorted and produced abnormal sterigmata and sporidia.

Manipulation. When the teliospores in the sori began to germinate, the moist chamber was removed from the Petri dish and covered with a cover glass. The chamber, thus completed, was then mounted under the microscope on the regular mechanical stage which had previously been moved somewhat to the right to prevent the chamber, when being mounted, from coming into contact with the end of the capillary tube. The capillary tube was then removed from its position and filled about two-thirds full with distilled water. This was done by releasing the end of the rubber tubing from the spring clamp and then by drawing the thumb and index finger of the right hand along the tubing, thereby creating a partial vacuum in the tubing, while the capillary end of the tube was held by the left hand under water. When the desired amount of water was drawn up, the spring

clamp was replaced and the tube mounted again in its former position, the capillary end of the tube appearing at the edge of the microscopic field (Fig. 3).

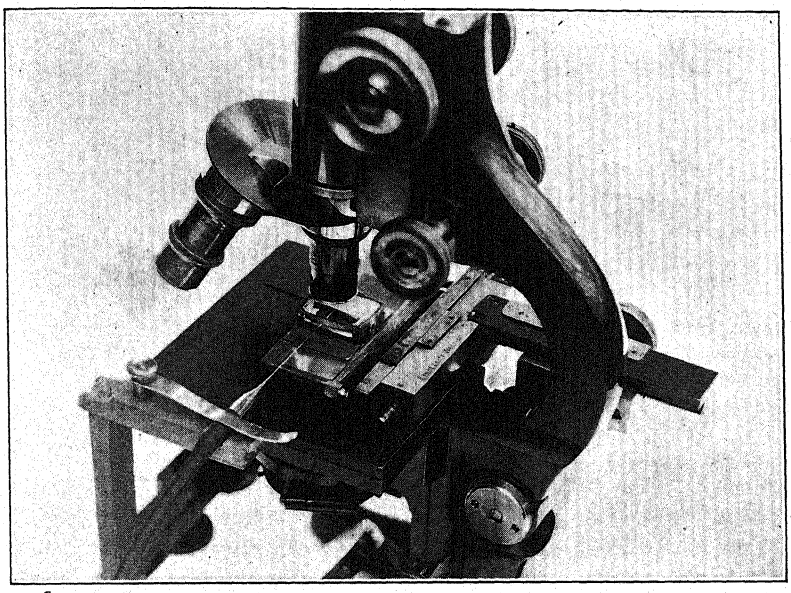


FIG. 3. View of middle portion of microscope showing moist chamber (cover glass removed) and capillary tube in position. The end of the capillary tube has passed through the rectangular opening of the moist chamber and has approached the mount of telium-bearing straw which is supported by the wooden pin.

By means of the mechanical stage, the moist chamber was moved to the left until the piece of straw bearing the telia appeared at the side of the field of view opposite that occupied by the end of the capillary tube, the latter having been adjusted for height so that it passed freely through the rectangular opening in the side of the chamber (Fig. 3). Search was then made for a somewhat isolated basidium bearing a sporidium which was approaching maturity. When one was found, it was brought directly opposite the end of the capillary tube (Fig. 4, A), but not close up to it, so that other sporidia which might be discharged while this one was maturing would not come into contact with the tube. If by any chance an unwanted sporidium did come into contact with the tube, the tube was removed, sterilized in boiling water, refilled with distilled water, and put back into position again.

Whenever the drop of water which forms at the base of the sporidium first became visible, the mount and capillary tube were moved towards one

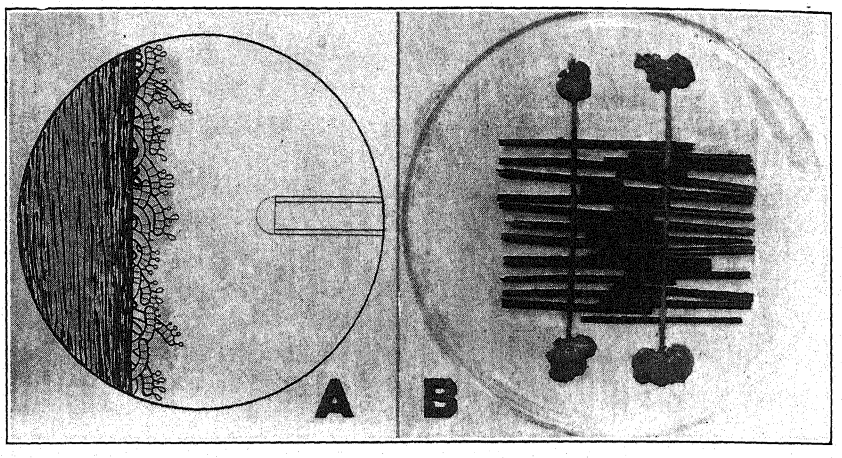


FIG. 4. A. Diagrammatic representation of a microscopic field showing the end of the capillary tube approaching a mature sporidium. The dotted half circle represents the hemispherical globule of water that comes into contact with the sporidium. B. Inside of a Petri-dish cover showing the method of mounting telial material of *Puccinia graminis* for inoculation purposes. Reduced.

another so that the sporidium and the end of the tube were separated by a very small distance. A light pressure on the rubber tubing caused the water in the tube to bulge out in hemispherical form, just far enough to establish contact with the sporidium. On release of the pressure, the water went back into the tube, carrying with it the sporidium.

The mount was drawn back as quickly as possible by a reverse movement of the mechanical stage to prevent contamination of the tube by other sporidia. The tube was removed, and, by a light pressure on the rubber tubing, a droplet of water containing the sporidium was deposited on a barberry leaf. The plant was then placed in an incubation chamber for 48 hours. At the end of that period, it was set on a bench in the greenhouse. After each inoculation, the capillary tube was sterilized in boiling water.

If a bisporidial inoculation was to be made, the process was repeated, and the two sporidia were deposited on the leaf in the same drop.

Generally, the second and third sporidia on a basidium mature first, and at about the same time; later, the one towards the tip; and, finally, the one situated nearest the teliospore. The latter one not infrequently aborts and collapses before it reaches maturity.

The comparative failure of this method of inoculation seemed to be due chiefly to physical causes. The waxy cuticle of a barberry leaf has little or no affinity for a droplet of water, so that, when one was placed on a leaf, it remained in almost spherical form, as if placed on an oily surface. It seems probable that the sporidium was held in the drop of water and

either did not germinate or, if it did germinate, its germ tube had little opportunity of establishing contact with the leaf.

(2) *Method B*

This method was much simpler. Petri dishes containing sufficient water to cover the bottoms were used as germination chambers for the teliospores of *Puccinia graminis* and *P. helianthi*.

Inoculations with *Puccinia helianthi* were made as follows. Pieces of rusted sunflower leaves were soaked in water for an hour. Some Petri-dish covers were then lined with thin pads of filter-paper. The leaves were placed in the Petri-dish covers so that their upper sides were in contact with the filter-paper, while their lower sides, which bore the telia, were turned upwards and exposed to the air. The moisture caused the filter-paper to adhere to the covers, and the leaves, in turn, to the filter-paper; so that, when the covers were set on their respective dishes, both the filter-paper and the pieces of leaves remained in position.

If inoculations with *Puccinia graminis* were to be made, the procedure was similar, except that the rusted pieces of culms were supported by short pieces of twine (Fig. 4, B), the ends of which were made fast to the covers by means of sealing wax.

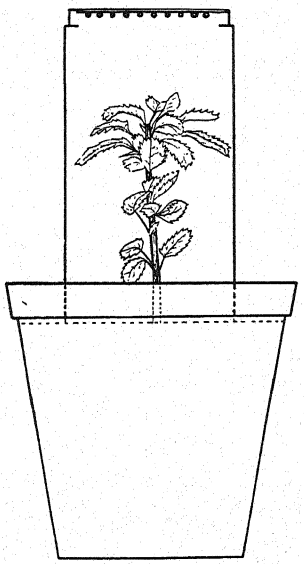


FIG. 5. Semi-diagrammatic drawing to show a barberry plant being inoculated by method B (Fig. 4). The small circles at the top represent short pieces of straw bearing rust sori. The pieces of straw are attached to the inner side of a Petri-dish cover which rests on the end of the hollow cylinder surroundings the plant.

With both *Puccinia graminis* and *P. helianthi*, the same method of inoculation was employed. The plant to be inoculated was first covered with a fine film of water. Over it was inverted a hollow cylinder (Fig. 5), at the upper end of which was held a Petri-dish cover bearing the leaves on which the sporidia were developing. The cover was kept continually in motion by a circular movement of the hand. The cylinder prevented air currents from carrying the sporidia away from the plant, and the movement of the cover insured an even distribution of the sporidia over the leaf surface. After inoculation, the plant was placed in an incubation chamber for 48 hours. At the end of that period, it was placed on a bench in the greenhouse.

As a rule, the sporidia settled on a leaf at some distance apart; but, sometimes, two of them settled close together. The sporidia were not actually seen on the leaves after they had settled, but their location was inferred from the position of the infections to which they gave rise (Fig. 6).



FIG. 6. Under side of a portion of a sunflower leaf showing two neighboring monosporidial pustules shortly after they appeared. These two simple pustules will soon coalesce to form a compound pustule. ×2.5.

The time required for each inoculation varied inversely with the number of sporidia which were being produced. An index of this number was obtained just before the inoculations began. A glass slide was placed in each Petri dish directly below the telia and was left there for 10 minutes. Thereafter, each slide was examined under the microscope, the number of sporidia that had settled on it was noted, and then the time necessary for

an inoculation was estimated and marked on the outer surface of the cover of the Petri dish. If the sporidia were relatively numerous, the time during which it was necessary to expose the leaves to the falling sporidia was short; if relatively scarce, the time was longer. Usually the time varied from 2 to 5 minutes.

EXPERIMENTAL RESULTS IN 1926

During the year 1926, inoculations were made exclusively by Method A. By it two monosporidial pustules of *Puccinia graminis* were obtained on barberry leaves. One of these appeared on a young leaf on July 9. It developed readily and eventually attained a diameter of 6 millimeters. The other one occurred on a comparatively old leaf on August 3. It grew less rapidly and scarcely attained half the diameter of the first one. Both pustules, however, developed pycnia which exuded nectar containing numerous pycnispores. But, although both pustules remained healthy for 5 weeks, neither of them produced aecia. A cytological examination of each pustule, when 5 weeks old, showed that its mycelium was still in the haploid condition.

Three of the bisporidial inoculations by this method were successful. On one of the leaves inoculated on August 5, two neighboring infections occurred. These appeared first as tiny, pale yellow pustules, approximately 2 mm. apart. About 6 days later, the two pustules coalesced. Five days after coalescence, aecia began to appear in the compound pustule thus formed. On August 17, two other barberry leaves were successfully inoculated. Two pustules, about 2 mm. apart, developed on each leaf and coalesced 7 to 8 days later. Aecia appeared in one of the compound pustules so formed within 6 days, but none appeared in the other one within that time or thereafter.

These results, although few in number, were of considerable significance, for they at least indicated that the mycelia of the pustules, and consequently the sporidia from which the mycelia originated, were of two sexes and that in all probability *Puccinia graminis* is heterothallic. They also were indicative of the behavior that might be expected of pustules in further experimentation.

EXPERIMENTAL RESULTS IN 1927

So far in the investigation, *Puccinia graminis* was employed; but, as the teliospores of this rust begin to germinate rather late in the spring—towards the end of April in Canada—it was decided to select another rust, one in which teliospore germination occurs earlier, for the preliminary investigational work of 1927. *P. helianthi* was chosen. Its teliospores were known to germinate earlier in the spring than those of *P. graminis*, and an

abundance of telial material was easily procurable. Moreover, as the writer wished to try inoculation Method B, this rust seemed well adapted to this purpose. In order to have telial material available, a liberal supply of rusted sunflower leaves was collected late in the autumn of 1926 and stored in the basement of the laboratory.

Towards the end of February, 1927, the teliospores of *Puccinia helianthi* began to germinate. Sunflower seedlings were inoculated by sowing sporidia sparsely over the leaves, as already described. Infections became manifest about 8 days after inoculation as tiny red pustules on the leaves. Very frequently the pustules were isolated, often only one on a leaf, or two or three rather widely separated from one another on the same leaf. Less frequently, two pustules arose relatively close together, from 1 to 4 mm. apart, and later coalesced to form a compound pustule. Occasionally three or four pustules occurred in a cluster, but these were discarded forthwith.

As soon as the pustules became visible, the position of the pustule, or pustules, on each leaf was mapped on a label which was then attached to the leaf. In this way it was possible to determine, when the pustules became older, which pustules were simple in origin (monosporidial) and which were compound in origin (bisporidial). If the distance between two neighboring pustules was greater than 4 mm. each one was considered as a simple pustule. Usually two neighboring pustules were sufficiently far apart when they first appeared to be readily distinguishable from each other (Fig. 11); but, occasionally, it was difficult to decide whether or not the infection giving rise to a pustule was of monosporidial or of bisporidial origin. In these cases, which fortunately were not numerous, the pustules were considered as monosporidial in origin.

In the course of the investigation, many sunflower seedlings were inoculated and, as a result of these inoculations, a large number of pustules were made available for study. On account of the uniformity of technique and the similarity of results in all the earlier experiments, it is unnnecessary to describe the experiments in consecutive order or to record separately the data for each one. It will suffice to give a general descriptive statement concerning the development of both simple and compound pustules, and a summary of the experimental results obtained with each type.

Evidence of heterothallism in Puccinia helianthi. The simple (monosporidial) pustules of *Puccinia helianthi* developed vigorously. A few of them finally attained a diameter of 12 mm., but usually the diameter was much less, from 6 to 8 mm. Pycnia developed plentifully on the upper side of all the pustules and less numerously (Fig. 7), or not at all, on the under side. When they were present on the under side, they were gen-

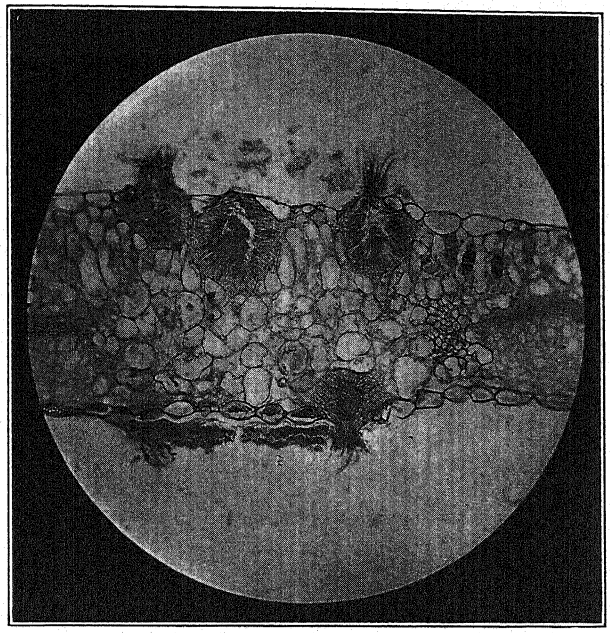


FIG. 7. Section through a part of a monosporidial pustule of *Puccinia graminis* on a barberry leaf showing pycnia present on both sides of the pustule. Magnification, 100. From a photomicrograph by W. F. Hanna.

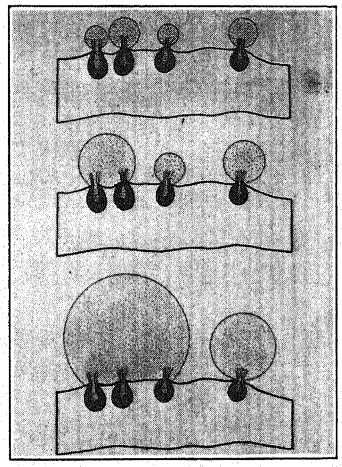


FIG. 8. Diagrammatic representation of a vertical section through a barberry leaf to show how the globules of nectar produced by the pycnia enlarge and finally fuse to form a layer of nectar over the whole surface of the pustule. The small dots in the globules represent pycniospores.

erally most numerous in the peripheral region of the older pustules, many of which were beginning to die at the center. Nectar, containing numerous pycniospores, was exuded by the pycnia of both sides of the pustules. If the pycnia were relatively close to one another, as on the upper side of the pustules, the globules of nectar, as they grew larger, came into contact and fused, so that eventually the whole surface of the pustule was covered with a layer of nectar. The process is illustrated diagrammatically in figure 8.

The majority of the simple pustules never produced aecia (Fig. 9, A), although some of them remained alive for upwards of 5 weeks. Aecia, however, appeared in a minority of them. Out of a total of 2,153 simple pustules, 1,641 went through the whole course of their development without producing aecia. The remainder, 512 in all, at one time or another developed aecia.

No regularity or periodicity marked the appearance of aecia in the latter pustules. Aecia might appear in one of them at any time after it was

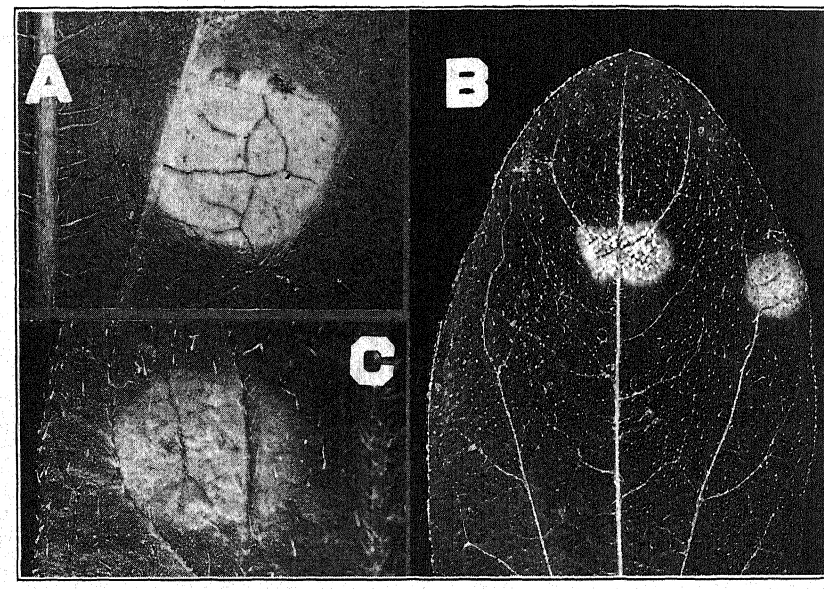


FIG. 9. A. Under side of a portion of a sunflower leaf showing a monosporidial pustule of *Puccinia helianthi* which has not produced aecia. A few pycnia (dark specks) are seen scattered over the surface of the pustule. Photographed 33 days after inoculation. ×5. B. Under side of a sunflower leaf showing a compound pustule of *P. helianthi* astride the midrib, with aecia, and a monosporidial pustule without aecia at the right-hand edge. Photographed 16 days after inoculation. ×1.75. C. Under side of a portion of a sunflower leaf showing a compound pustule of *P. helianthi* without aecia. Photographed 24 days after inoculation. ×4.

10 or 12 days old up to the time of its death, 3 or 4 weeks later. The sudden and spontaneous change from the haploid to the diploid condition took place without any apparent cause, thereby simulating a parallel phenomenon in the Hymenomycetes.

Each compound pustule came into being as a result of the coalescence of two neighboring simple pustules. The time necessary for two such pustules to accomplish coalescence depended largely on the distance they were separated. If the two pustules were about 1 mm. apart, they coalesced early, within 2 or 3 days after they appeared; if they were more widely separated, they coalesced later, possibly from 8 to 10 days after they appeared; but, if they were more than 4 mm. apart, they rarely coalesced. Prior to coalescence, each of the two pustules developed independently as a simple pustule. Following coalescence, one of two things happened. Either aecia appeared in the compound pustules thus formed within from 5 to 6 days (Fig. 9, B) or they did not appear (Fig. 9, C). Of the 246 compound pustules of *Puccinia helianthi* that came under observation, 108 developed aecia within from 5 to 6 days, while in the 138 others no aecia appeared within that time. However, some of these 138 pustules produced aecia at one time or another afterwards, so that, by the time all of the 246 pustules had died, those that had developed aecia had increased from 108 to 145, leaving 101 that had failed to produce aecia.

Evidence of heterothallism in Puccinia graminis. Experiments similar to these just described were made with *Puccinia graminis,* and similar results were obtained. As the barberry plants that were suitable for inoculation purposes were somewhat limited in number, the pustules available for observation were fewer than those of *P. helianthi.* Both simple and compound pustules appeared. Each pustule arose as a tiny pale yellow spot on the leaf. The difficulty in determining whether certain pustules were of monosporidial or of bisporidial origin was again encountered. Possibly, owing to the greater resistance offered by the leaf tissue of the barberry to the radial advance of the mycelium, the pustules of *P. graminis* did not grow so rapidly, or become so large, as those of *P. helianthi.* Very few of them exceeded 5 mm. in diameter. On account of this rather restricted growth, coalescence of pustules 4 mm. apart rarely occurred. On the other hand, the pustules of *P. graminis* produced nectar more copiously than did the pustules of *P. helianthi,* and they possessed greater longevity. The sunflower leaves aged more quickly than the barberry leaves, and the earlier aging of the *P. helianthi* pustules seemed to be attributable more to the decreased vigor of the leaves than to any lack of vitality on the part of the organism.

The data concerning the simple pustules of *Puccinia graminis* may be summarized as follows. Ten days after the first appearance of the pustules,

the number of simple pustules in which aecia had developed and the number in which aecia had not developed were recorded. Out of a total of 174 simple pustules on which observations were made, 11 produced aecia within that time, while 163 were without aecia (Fig. 10, A). From time to time one or another of these pustules developed aecia, so that, when practically all the pustules had died, it was found that altogether 37 of the simple pustules had produced aecia and 117 had failed to produce aecia. The apparently spontaneous change from a haploid to a diploid condition of the mycelium, as evidenced by the production of aecia in a certain number of the pustules of monosporidial origin, thus was observed in *P. graminis,* just as in *P. helianthi.*

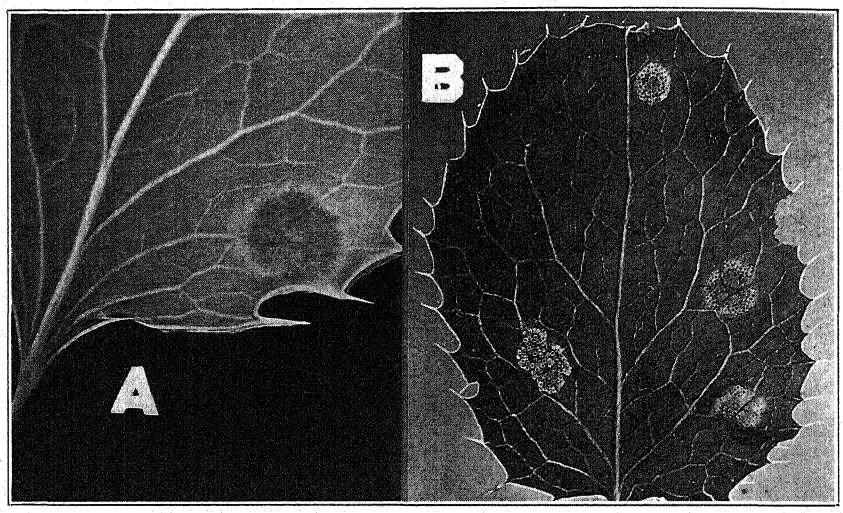


FIG. 10. A. Under side of a portion of a barberry leaf showing a monosporidial pustule of *Puccinia graminis* without aecia. Photographed 23 days after inoculation. ×3. B. Under side of a barberry leaf showing one compound pustule of *P. graminis* with aecia on the left of the midrib and three monosporidial pustules without aecia on the right of the midrib. For the peculiar pocked appearance of the three right-hand pustules *vide* the text. Photographed 20 days after inoculation. ×2.

In the compound pustules 5 or 6 days after coalescence had been effected, a record was made of those pustules that had produced aecia and of those that had not produced aecia. In a few of these pustules, coalescence was not thoroughly accomplished at that time. The number in which aecia were present was 24 and the number in which aecia were not present was 35. At the termination of the experiments, when most of the pustules had died, the proportion was 36 with aecia and 23 without aecia.

Figure 10, B, shows the under side of a barberry leaf, photographed 20 days after it was inoculated. On the left-hand side of the midrib appears a

compound pustule bearing aecia. On the right-hand side are three simple pustules in which no aecia developed. The pocked appearance of these three pustules is due to the formation of wefts of haploid hyphae which develop in simple pustules just beneath the epidermis and simulate in general contour a young aecium.

Mention was made of these structures by Olive (59) in 1911. He believed them to be sterile aecia. They were observed by the writer (15) in 1928. Hanna (29), in 1929, referred to them as being "evidently haploid rudiments of aecial cups waiting to be stimulated into further developmental activity." In the same year, Miss Allen (1) stated that they resemble aecia but "consist of haploid mycelium only." As this paper is not primarily concerned with the cytological aspect of the problem, no further reference will be made to these haploid hyphal wefts.

The results thus far enumerated indicate that *Puccinia graminis* and *P. helianthi* are heterothallic and that the mycelium of an individual pustule is either (+) or (−) in its sexual nature, so that, when a (+) pustule and a (−) pustule coalesce, a sexual interaction takes place between the two mycelia which results in the production of aecia; but that, when two pustules of the same sex coalesce, *i.e.,* a (+) pustule with another (+) pustule or a (−) pustule with another (−) pustule, no sexual interaction occurs and no aecia are produced.

Although heterothallism accounts for the behavior of the majority of the simple compound pustules in *Puccinia graminis* and *P. helianthi,* it gives no explanation of the apparently spontaneous change from the haploid to the diploid condition in simple pustules, or in compound pustules which have long remained sterile. A partial explanation, at least, of this behavior was brought to light when the function of the pycnia was discovered.

Before treating of the individual experiments which deal with the function of the pycnia, it might be pointed out that in each experiment the treated pustules and those kept as a control were of the same age and of comparable vigor. All of the pustules were of monosporidial origin: they bore numerous pycnia, but aecia were entirely absent from them. At the beginning of each experiment, the minimum age of the *Puccinia graminis* pustules was 16 days and of the *P. helianthi* pustules, 14 days. Wherever the experimental technique demanded it, the instruments used were thoroughly sterilized in an alcohol flame.

The effect of mixing nectar. In one experiment with *Puccinia helianthi,* the nectar of 184 simple pustules was intermixed by means of a small scalpel, so that the nectar of each pustule was distributed over the surface of several other pustules. This procedure may have caused a slight irritation of the upper surfaces of these pustules; and, therefore, to procure comparable conditions in 174 other similar pustules, which served as a control,

the nectar of each of these pustules was stirred separately with the scalpel but not mixed with any other nectar.

Five days after the beginning of the experiment, the condition of the pustules was as follows: of the 184 pustules in which the nectar had been mixed, 176 had produced aecia, 4 no aecia, and 4 had wilted and died through leaf injury; of the 174 pustules in which the nectar had been stirred but not mixed, only 20 had produced aecia, while 154 were entirely free from aecia (*cf.* Fig. 11).

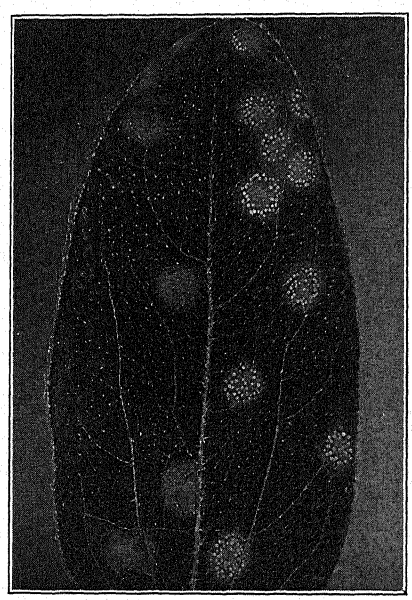


FIG. 11. Under side of a sunflower leaf showing monosporidial pustules of *Puccinia helianthi* on either side of the midrib. Twenty days after inoculation, all the pustules were free from aecia. At that time, the nectar of the pustules on the right of the midrib was well mixed; while, as a control, the nectar of the pustules on the left of the midrib was stirred separately but not mixed. The right-hand pustules have all developed aecia, but the left-hand pustules show no aecia whatsoever. Photographed 6 days after the mixing was done. ×1.5.

An experiment similar to that just described was made with *Puccinia graminis*. The nectar on the upper surface of 116 simple pustules was intermixed; while, as a control, the nectar of 85 other similar pustules was stirred separately but not mixed with any other nectar.

Six days after the experiment had begun, the condition of the pustules was as follows: of the 116 pustules in which the nectar was mixed, 102 had produced aecia and 14 no aecia; whereas, of the 85 pustules in which the nectar was stirred but not mixed, 17 had produced aecia, while 68 were free from aecia (*cf.* Fig. 12, A).

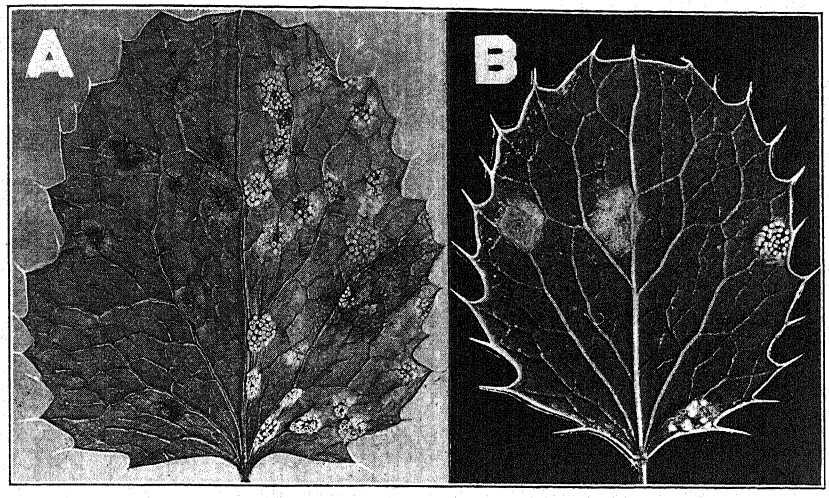


FIG. 12. A. Under side of a barberry leaf showing monosporidial pustules of *Puccinia graminis* on either side of the midrib. Thirteen days after inoculation, one pustule, on the right of the midrib, produced aecia. All of the others were free from aecia 17 days after inoculation. At that time, the nectar of the pustules on the right of the midrib was well mixed; while, as a control, the nectar of the pustules on the left was stirred separately but not mixed. All the pustules on the right-hand side of the leaf have developed aecia, but the left-hand pustules show no aecia whatsoever. Photographed 9 days after the mixing was done. ×2. B. Under side of a barberry showing 4 monosporidial pustules of *P. graminis*. Sixteen days after inoculation, the pustules were free from aecia. At that time nectar heated for 3 hours at 70° C. was applied to the upper surface of the 2 pustules on the left of the midrib, while unheated nectar was applied to the upper surface of the 2 pustules on the right of the midrib. The right-hand pustules alone have developed aecia. Photographed 8 days after the nectar was applied. ×1.5.

The rôle played by flies. Proof that flies mix the nectar of separate monosporidial pustules and so cause the mycelia of the pustules to change from the haploid to the diploid phase, as shown by the appearance of aecia, was obtained in an experiment with *Puccinia helianthi*.

Fifteen to 20 flies were enclosed in a large screen-wire cage with 12 pots of sunflower seedlings on the foliage leaves of which there were 98 simple pustules bearing pycnia but no aecia. As a control, 159 similar pustules on the foliage leaves of sunflower seedlings were protected by a screen-wire cage from the visits of flies.

Eight days after the beginning of the experiment, 96 of the 98 pustules to which flies had access had produced aecia and only 2 no aecia; whereas only 5 of the 159 pustules to which flies had not had access had produced aecia.

It seems very probable that many other insects are active agents in bringing about the transfer of nectar from one pustule to another. Rathay (63) states that he observed 135 different species of insects, belonging to the Coleoptera, Hymenoptera, Hemiptera, and Diptera, visiting pustules of two Gymnosporangium species and also pustules of the common cereal rusts. Shear (68), Grove (25), Meineke (48), and Spaulding (70) also have pointed out that insects are attracted by the nectar.

That insects visit rust pustules is not surprising. It is known that the pycnial nectar of many rusts is sweetish to the taste and that in some rusts the pycnia have a distinct odor. Rathay (63), by chemical tests, showed that the nectar of *Gymnosporangium sabinae* (Dicks.) Wint. contained dextrose and levulose. The odor of the pycnia of certain rusts has been mentioned by Persoon (60), Tulasne (80), Léveillé (39), and Rathay (63). Plowright (61) pointed out that these odors were possibly to attract insects, mimicking as they do the perfume of flowers. No odor has been detected by the writer in *Puccinia graminis* and *P. helianthi,* but he has found that the nectar of the former is distinctly sweetish to the taste.

Effect of heating the nectar. By another experiment it was found that heating the nectar of *Puccinia graminis* or of *P. helianthi* to a temperature of 70° C. for 3 hours to kill the pycniospores rendered the nectar ineffective in inducing the production of aecia when it was applied to the pycnia of individual simple pustules.

Nectar was collected from approximately 50 pustules of *Puccinia graminis* by means of short capillary tubes. One tube was used for each pustule. The nectar was then deposited in a single drop, mixed thoroughly, and sucked up into 2 fine glass tubes, each tube taking up approximately the same amount of nectar. One end of each tube was stopped with sealing wax and the other by a plug of cotton wool. One of the tubes was placed in an oven and kept for 3 hours at 70° C.; the other was kept at room temperature for the same length of time. The heated nectar was then allowed to cool to room temperature, and a small quantity of it was then deposited on each of 25 simple pustules. The droplet on each pustule was mixed thoroughly with the nectar of the pustule to which it was applied. The nonheated nectar was similarly distributed among 20 other similar pustules of *P. graminis.*

Six days later, the condition of the pustules was as follows: of the 25 pustules to which the heated nectar was applied, only 1 developed aecia, while 24 were entirely free from aecia; of the 20 pustules to which nonheated nectar was applied, 17 developed aecia, whereas only 3 were free from aecia.

Figure 12, B, illustrates the results. The pustules on the left of the midrib, to which heated nectar was applied, have produced no aecia; those

to the right of the midrib, to which nonheated nectar was applied, have produced aecia.

A similar experiment was made with *Puccinia helianthi.* Heated nectar was added to 27 simple pustules and nonheated nectar to 25 other similar pustules. At the end of 5 days, aecia were completely absent in 23 of the 27 pustules to which the heated nectar was added, 4 only having formed aecia; while of the 25 pustules to which the nonheated nectar was added, all produced aecia.

These two experiments indicate that the pycniospores are the effective agents in inducing the formation of aecia, and not the nectar. The evidence, however, is not absolute. It is recognized that if the agent were an enzyme in the nectar the heating might destroy its activating property.

Sex of pycniospores. Another experiment was designed to show whether or not the pycniospores of *Puccinia graminis* are of two kinds, (+) and (−). The nectar of one monosporidial pustule of *P. graminis* was drawn off by means of a capillary tube and divided into several drops and then the drops were applied singly to the pycnia of as many pustules as there were drops. The nectar of other simple pustules was divided in like manner and distributed among other pustules. Altogether, 74 individual pustules were thus treated. Six days later, 30 of the 74 pustules had developed aecia, while 44 were free from aecia. As a control, 26 similar pustules were left untreated. Aecia appeared in only one of these.

Figure 13, A, 22 is illustrative of the results. Nectar from one pustule was applied to each one of the four pustules. Aecia arose in two of them but not in the other two. Usually, however, among pustules on the same leaf, the ratio of those with aecia to those without aecia was less evenly balanced.

The experiment just described was repeated with *Puccinia helianthi.* Five days after the drops of nectar were applied, 15 of the 48 pustules treated had developed aecia, whereas 33 of them were without aecia. In a control of 66 similar pustules, only 5 produced aecia within that time.

Theoretically, if each basidium bears two (+) sporidia and two (−) sporidia, the number of pustules with aecia and the number without aecia should be equal in each experiment. The ratio obtained with the pustules of *Puccinia graminis* is possibly as near the theoretical ratio as could be expected with such a small number of pustules. With the pustules of *P. helianthi,* there is a much greater divergence, yet not so great as appears in the control. The rather indifferent ratio given by the pustules of *P. helianthi* is very probably attributable, in some way or other, to the meager quantity of nectar that was being produced by pustules at the time the experiment was performed.

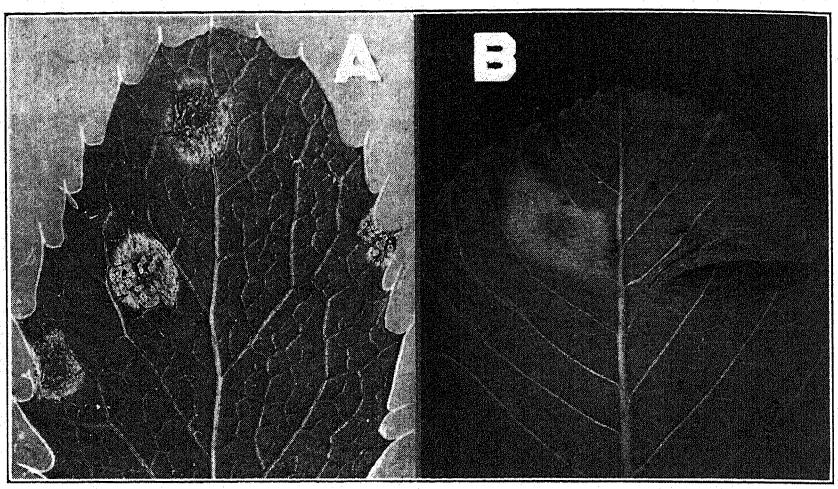


FIG. 13. A. Under side of a barberry leaf showing 4 monosporidial pustules of *Puccinia graminis*. The nectar from a monosporidial pustule on another leaf was divided into 4 small drops and added to the 4 pustules, 1 drop to a pustule, when they were 17 days old. Within 5 days aecia arose in 2 of them but not in the other 2. The photograph was taken 10 days after the nectar was added. ×2. B. Under side of a leaf of *Amelanchier alnifolia* showing a pustule of a *Gymnosporangium* sp. (*corniculans?*) which was 6 weeks old at the time the photograph was taken. The pustule failed to develop aecia. ×2.5.

Aecia in monosporidial pustules. It will be observed that in all the experiments so far discussed aecia developed apparently in a spontaneous manner in at least some of the monosporidial pustules. When the effect of mixing the nectar of pustules of opposite sex was discovered, it was thought that possibly mixing of nectar had taken place by some unknown means and that, under carefully controlled conditions, few or none of the pustules would develop aecia. It is to be noted that in the earlier experiments: (1) the pustules were examined frequently, and there was the possibility that, in the repeated handling of the leaves bearing pustules, nectar was transferred from one pustule to another; and that (2) no protection was afforded the pustules from the visits of flies or other insects that might by chance have been in the greenhouse.

A more critical experiment than any of those already recorded was devised with a view to preventing the accidental intermixing of nectar.

Sunflower seedlings were inoculated in the greenhouse by allowing sporidia of *Puccinia helianthi* to fall sparsely onto the upper surface of the first two foliage leaves when they were about one inch in length. Usually, about six seedlings grew in a pot; but, whenever the minute pustules appeared, each plant showing infection was planted by itself in a separate pot

and covered with a screen-wire cage. By keeping each plant in an individual cage, the opportunity for the transfer of nectar from one pustule to another was reduced to a minimum. All uninfected leaves were removed, and no new ones were allowed to develop.

Only pustules that were entirely free from aecia at 17 days of age were selected for observation. Of these there were 228, distributed as follows: 93 pustules were borne singly on as many plants; the remaining 135 were borne on 59 other plants all of which bore at least 2 pustules, usually 1 on each leaf, but a small number of the plants bore 3 pustules, and a very few, 4 pustules.

Not all of the pustules persisted for the same length of time. Some of them died when about 4 weeks old and most of them before they were 6 weeks old, but a few were still living and exuding nectar in their peripheral region when 7 weeks old—when the final data were recorded. Within the 7 weeks, aecia developed in 11 of the 228 pustules. Of the 93 pustules, each of which was borne on a separate plant, only 2 produced aecia. Of the 135 pustules, of which 2 or more were borne on individual plants, 9 produced aecia. Altogether 217, or 95 per cent, remained free from aecia during the whole course of the experiment.

From the time the pustules first appeared, daily inspections were made of the plants in order to destroy any insects that might have gained access to them. White flies (*Aleyrodes*) and thrips (*Heliothrips*) were the only ones discovered, and then only infrequently. The former were rarely found in contact with the pustules, but the latter seemed to be attracted by the nectar and fed upon it in preference to the leaf tissue. Consequently, in spite of the precautions taken, there was the possibility that thrips carried the nectar of one pustule to the pycnia of another, especially on leaves that bore two or more pustules. This assumption is supported by the fact that nine of the pustules that produced aecia occurred on leaves bearing two or more pustules, and only two on plants bearing but a single pustule.

If, in the experiment just described, there had been an absolute exclusion of insects and a complete absence of any means by which the transfer of nectar could have taken place, it is possible that no aecia whatever would have developed in any of the pustules. At present, it is impossible to say that aecia never develop spontaneously in simple pustules of *Puccinia helianthi;* but it has been clearly shown that, under the conditions of the experiment, the percentage of pustules that actually produced aecia was extremely small, not more than 5 per cent. The experiment was not repeated with pustules of *P. graminis,* but later observations showed that, where monosporidial pustules of this rust were protected from the visits of insects, very few of them developed aecia.

Occurrence under natural conditions of pycnia unaccompanied by aecia. So far as the writer is aware, it is not recorded that pustules of monosporidial origin occur in nature, although undoubtedly such pustules have been seen by other observers and mistaken by them for young pustules in which aecia had not yet developed. Observations made during the summer of 1927 and 1928 have revealed that, in nature, pycnia are frequently unaccompanied by aecia in the following rusts: *Puccinia graminis* on *Berberis vulgaris* var. *purpurea; P. coronata* on *Rhamnus cathartica* L.; *P. Pringsheimiana* on *Ribes grossularia* L. (cultivated and wild); and a *Gymnosporangium* sp. (possibly *corniculans* Kern.) on *Amelanchier alnifolia* Nuttall (Fig. 13, B).

Leaves of these hosts bearing young and apparently monosporidial pustules were marked by means of a small tag as soon as the pustules were noticed. When the pustules were about 14 or 15 days old, those showing no signs of aecia were selected for further observation. It was thought that pustules of this age could not include any compound pustules of two mycelia of opposite sex, for compound pustules of this type would have produced aecia when several days younger.

At intervals during the next 3 or 4 weeks, the tagged pustules were examined. Within that time, some of the pustules produced aecia, but others did not. A few of the pustules persisted for a week or more longer, but none of these bore aecia. The results for 1927 are summarized in table 1.

TABLE 1.—*Summary of observations made in 1927 on the occurrence in nature of aecia in monosporidial pustules*

Name of rust	Period of observation (in weeks)	Total number of pustules	Pustles at end of period	
			Number with aecia	Number without aecia
Puccinia graminis	4½	50	37	13
P. coronata	2½	61	45	15
P. Pringsheimiana	3	60	16	44
Gymnosporangium sp.	3	60	52	8

It should be noted that none of these pustules were protected in any way from the visits of insects, and there is little doubt that insects did actually transfer nectar from one pustule to another, as insects of various kinds were seen flying about, or crawling over, the leaves of the host plants, and some of them were even observed in contact with the pustules. However, it also should be mentioned that, on account of the excessive precipitation, the pustules were frequently washed, and only very seldom was

there any noticeable amount of nector available for transfer. Perhaps the reduction in the amount of available nectar accounts for the failure of so many of the pustules to produce aecia, despite the activity of the insects.

During the summer of 1928, young pustules, apparently monosporous in origin, were again marked, but, instead of leaving them exposed, as was done in the previous year, most of the leaves bearing them were covered with one layer of white cheesecloth in order to prevent insects from visiting the pustules. This protection excluded most of the winged insects but was less effective against ants and small spiders.

Not all the pustules marked were found as soon as they appeared. At the time the coverings were applied, some pustules were at least 1 week old; most of them, however, were just appearing or were not more than 2 or 3 days old. Unfortunately, the pustules available for study were much less numerous this year than in the previous year. There were 20 pustules of *Puccinia graminis* on *Berberis vulgaris* var. *purpurea,* 2 pustules of *P. coronata* on *Rhamnus cathartica,* and 51 pustules of *P. Pringsheimiana* on *Ribes grossularia.* No pustules of the *Gymnosporangium* sp. occurred on *Amelanchier alnifolia.*

About one-third of the pustules on Ribes were left unprotected to serve as a check for the protected ones and for comparison with the data collected in 1927. The results for 1928 are summarized in table 2.

TABLE 2.—*Summary of observations made in 1928 on the occurrence in nature of aecia in monosporidial pustules*

Name of rust	Covered or uncovered	Period of observation (in weeks)	Total number of pustules	Pustules at end of period	
				Number with aecia	Number without aecia
Puccinia graminis	Covered	3	20	4	16
P. coronata	Covered	3	2	0	2
P. Pringsheimiana ...	Covered	3	33	6	27
P. Pringsheimiana ...	Uncovered	3	18	6	12

It will be seen from tables 1 and 2 that: (1) a much smaller percentage of the covered pustules in 1928 produced aecia than the uncovered ones in 1927; and (2) that, in 1928, less than 20 per cent of the covered pustules of *Puccinia Pringsheimiana* developed aecia, as compared with 50 per cent of those that were not covered. Apparently, by preventing the free access of insects to the pustules, the opportunity for transferring the nectar of one pustule to another was reduced, and consequently the number of simple pustules which remained free from aecia was augmented. It is not probable that the cheesecloth covering intercepted sufficient sunlight to

inhibit, or retard materially, the formation of aecia. This supposition is supported by the fact that aecia formed in a small number of the covered pustules. The development of aecia in these pustules was very probably induced through the transfer of nectar to them by insects before the coverings were applied.

From the fact that, under natural conditions, monosporidial pustules of *Puccinia coronata,* of *P. Pringsheimiana,* and of the *Gymnosporangium* sp. behave like monosporidial pustules of *P. graminis,* and, from the fact that *P. graminis* has been shown experimentally to be heterothallic, it may be inferred that *P. coronata, P. Pringsheimiana,* and the *Gymnosporangium* sp. also are heterothallic.

Aecia limited to one part of a pustule. While the data recorded above were being collected in the field, it was noticed that occasionally aecia were present in only one part of a pustule and not all over it. This phenomenon was observed in pustules of *Puccinia coronata, P. Pringsheimiana,* and *P. graminis* (Fig. 14, A). A plausible explanation of this behavior is that nectar from a pustule of opposite sex had been deposited on the upper surface of the pustule but only on the area under which the aecia had developed.

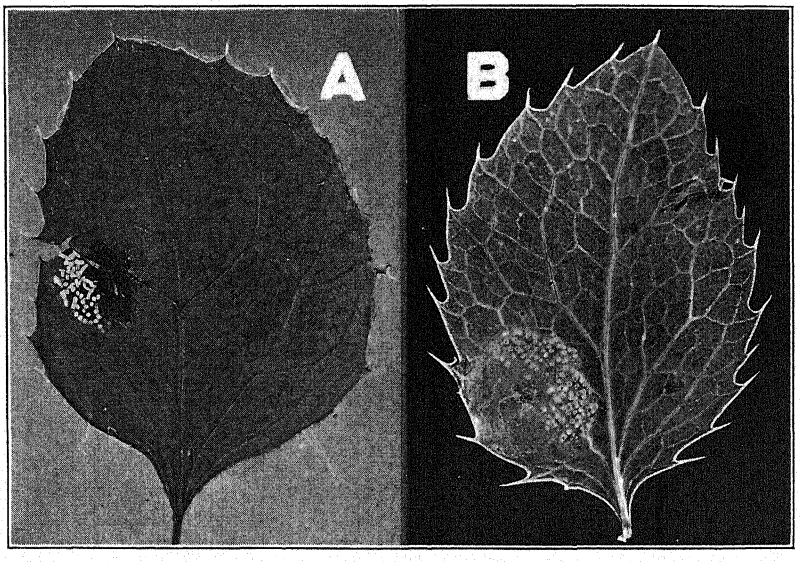


FIG. 14. A. Under side of a barberry leaf showing aecia present on the left-hand side, but not the right-hand side of a pustule of *Puccinia graminis,* which arose from an infection by natural inoculation. ×2. B. Under side of a barberry leaf showing a monosporidial pustule of *Puccinia graminis* to which, when 20 days old, a small quantity of composite nectar was applied to the upper surface of that part in which aecia are now seen, but the other part was left undisturbed. Photographed 7 days after the nectar was applied. ×2.5.

An attempt was made to reproduce experimentally similar results in monosporidal pustules of *Pucciana graminis*. Nectar was collected from about a dozen pustules and deposited on a glass slide in one drop. As (+) and (−) pustules were known to occur in about equal numbers, it was anticipated that in the drop both (+) and (−) pycniospores would be present. The drop was then stirred to distribute the two kinds of pycniospores evenly through it, and a small portion of this composite nectar was spread over one-half of the upper surface of a pustule that was 25 days old. Several other pustules of the same age were similarly treated. Before this nectar was added to a pustule, however, as much as possible of its own nectar was drawn off by means of a capillary tube in order that the nectar which was being added might be more definitely restricted to the area intended for it. Within 5 days, aecia developed in each pustule on the under side of the treated parts but not of the untreated parts (Fig. 14, B).

The experiment was repeated with monosporidial pustules of *Puccinia helianthi,* and similar results were obtained.

In most of the pustules, particularly in those of *Puccinia helianthi,* the aecia remained localized in the manner just described, but, in two or three pustules of *P. graminis,* there appeared to be a tendency for the development of aecia to extend subsequently from the treated half of the pustule, first to the adjacent untreated part, and finally to the remainder of the pustule.

The extension of aecial development just referred to may be explained by assuming that, in the course of a few days after the composite nectar was applied to the pustules, the pycniospores on the treated parts were carried slowly across the untreated parts by a flowing movement of the nectar; for the upper surfaces of the pustules were usually convex or concave, rarely flat; and day by day, as the pustules secreted more nectar, a state of flux was set up, and thus the composite nectar was brought into contact with the pycnia of the untreated parts. In support of this assumption, it may be mentioned that in pustules of *Puccinia helianthi* the amount of nectar exuded is much less than in pustules of *P. graminis* and that in *P. helianthi* aecial development was confined to the parts that received the composite nectar. Another possible explanation is that the mycelium of the treated part may have advanced gradually into the untreated part and there, in some way or other, have caused the development of aecia.

From the above it will be seen that the phenomenon of the occurrence of aecia in one part of a monosporidial pustule, as observed under natural conditions for *Puccinia coronata, P. Pringsheimiana,* and *P. graminis,* has been successfully reproduced in the laboratory in experiments made upon *P. graminis* and *P. helianthi.*

Origin of a new physiologic form. During the investigation, the aeciospores from some of the aecia that had been caused to develop by the application of nectar to the pycnia of pustules were handed over to Margaret Newton and Thorvaldur Johnson, of the Dominion Rust Research Laboratory, to inoculate wheat seedlings in order that the physiologic forms represented in these aecia could be identified. It was thought that, possibly in the telial material that produced the sporidia which led to the development of the pustules, two forms of rust might have been present and that, in some of the experiments, hybridization of two forms might have taken place. The form (or forms) present in the parental teliospores was not known; but it was argued that, if a physiologic form hitherto unknown could be identified by sowing the aeciospores on wheat and making cultural studies on the differential wheat varieties, its identification would be partial evidence at least that hybridization had occurred. On the other hand, there was the possibility that, if a new form was found, it might have been present in nature previously without having been discovered. However, as the survey for physiologic forms had been quite thorough in the vicinity of the Agricultural College, Winnipeg, where the telial material was collected, it was not very probable that an existing form had escaped detection. The likelihood was that, if a new form was isolated, it would be the product of a cross, or the result of segregation in a physiological form, a circumstance which, of itself, would be evidence of the hybrid nature of physiologic forms.

From one of the aecial cultures, a new and distinct physiologic form of stem rust was isolated. This form has been designated as No. 57, by Stakman and Levine.

RECENT WORK OF OTHERS ON HYBRIDIZING PHYSIOLOGIC FORMS

Since the investigation here described was carried out, confirmatory evidence that physiologic forms of *Puccinia graminis* do hybridize on the barberry has been obtained by several investigators. The crossing of physiologic forms was effected by the various investigators by transferring pycnial nectar in the manner described by the writer in 1927 and in an earlier part of this paper. Waterhouse (88), in 1929, reported that he had obtained two forms of rust in Australia from crosses made on the barberry. Newton, Johnson, and Brown (57), in 1929, working at Winnipeg, found that, when certain physiologic forms are selfed on the barberry, segregation takes place with the production not only of some old forms but also some new ones. The identification of these old and new forms indicated that the original forms must have been heterozygous for pathogenicity and therefore hybrid in their nature. They found one form that was homozygous. Furthermore, by making reciprocal crosses between forms, they obtained other forms which were apparently the products of direct crosses.

Stakman, Levine, and Cotter (74), in 1929, working at the University of Minnesota, reported successful crosses between *P. graminis tritici* form 36 and *P. graminis agrostidis*, from which they obtained forms that were different, in respect to pathogenicity, from both forms.

DISCUSSION

Experimental evidence from (1) observations on simple (monosporidial) and compound (bisporidial) pustules and from (2) observations on the effect of transferring pycnial nectar has been presented which shows that simple (monosporidial) pustules of *Puccinia graminis* and *P. helianthi* are of two kinds, (+) and (−), and that these two kinds are about equal in numbers. As a deduction, it follows that the mycelia of the pustules, and, therefore, the sporidia from which they originate, are of two different kinds, (+) and (−), and that these two kinds are about equal in numbers. This suggests that segregation of the (+) and (−) factors takes place in the basidium during the nuclear division in the same manner as it was found to take place in *Coprinus Rostrupianus* by Miss D. E. Newton (55) and in *C. radians* by Vandendries (84).

A cytological study of the mycelia of *Puccinia graminis* and *P. helianthi* has not been made; but, from the results obtained by sowing two sporidia of the same species close together on a leaf of the appropriate host, a theoretical explanation of what takes place may, for the present, be offered. It is as follows: When a (+) sporidium and a (−) sporidium are sown close together on a leaf so that the pustules arising from the two infections coalesce, the two monosporous mycelia come into contact, fuse together, and give rise to binucleate aeciospores, each conjugate pair of nuclei formed in the spore bed consisting of a (+) and of (−) nucleus derived from a (+) and a (−) mycelium, respectively. On the other hand, when two sporidia of the same sex, either two (+) or two (−) sporidia, are sown close together on a leaf so that the two pustules arising from the two infections coalesce, the two monosporous mycelia come into contact but do not interact sexually and consequently do not give rise to aeciospores.

The investigation has not finally disposed of the age-long question regarding the real nature of the pycniospores, yet it has produced some tangible evidence from which certain inferences may be drawn. In the first place, it has shown that pycnia develop in every pustule of monosporidial origin and that they all produce pycniospores; secondly, that (+) pycnia produce (+) pycniospores, and (−) pycnia, (−) pycniospores; thirdly, that the application of pycniospore-containing nectar taken from a (+) pustule to the pycnia of a (−) pustule, or *vice versa*, leads to the production of aecia in the pustule to which the nectar is applied; and, lastly, that, when the pycniospores in the nectar are killed by heat, the nectar loses this activating property.

Since pycniospores are produced by the pycnia of every monosporidial pustule, it is clear that, if the pycniospores are male gametes (spermatia), *Puccinia graminis* and *P. helianthi* are not dioecious. In other words, the monosporidial mycelia are not of two kinds: (a) male, bearing spermatia, and (b) female, not bearing spermatia.

Assuming that the pycnia are male conceptacles and that the pycniospores are gametes, it is logical to expect that female counterparts would also be present. Such counterparts, however, have not been discovered.[2] Their absence might be explained by their complete degeneration; but, in the evolutionary development of other fungi, in those forms where degeneration of the sexual organs occurs, it is the male elements that first undergo degeneration. If in the pycnium-producing rust fungi the female structures have entirely disappeared, these fungi occupy a wholly anomalous position among the fungi. Particularly is this true if the pycniospores perform some definite function. The evidence advanced in this paper indicates quite clearly that they do. Complete degeneration has then overtaken the female elements, while the male elements have retained their pristine vigor.

That the pycniospores are not male gametes seems most probable. The writer is inclined to regard the pycniospores as conidia corresponding to the uninucleate oidia of such heterothallic Hymenomycetes as *Coprinus lagopus, C. niveus, Stereopharia semiglobata,* and *Collybia velutipes.* In these species, oidia are produced by both (+) and (−) monosporous mycelia, and cell fusions between (+) and (−) mycelia initiate the diploid phase.

The pycnia may then be regarded, not as spermogonia producing nonfunctional spermatia, but as active organs which develop either (+) or (−) pycniospores and attract flies and other insects by means of which the pycniospores of one sex are carried to the pycnia of another sex. They occur chiefly on the upper side of leaves where they are readily accessible to insects, and they are usually red or yellow, by which means they are made conspicuous. Odors are emitted by the pycnia of some rusts and in many species the nectar is sweetish. These properties, no doubt, are advantageous

[2] This statement is no longer correct, and the opinion here expressed concerning the nature of the pycniospores is no longer tenable. Andrus (2) has found hyphae in *Uromyces appendiculatus* (Pers.) Fries and *U. vignae* Barclay that, in his opinion, are functionally the equivalent of trichogynes. The pycniospores function as spermatia. Recent unpublished results of research carried on by Ruth F. Allen, of the Bureau of Plant Industry, U. S. Department of Agriculture, show that in the gametophytic (haploid) generation of *Puccinia coronata* Corda, *P. graminis* Pers., and *P. triticina* Eriks. certain hyphae grow either to the ventral or to the dorsal surface of the host leaf by pushing into stomata or between epidermal cells, or even by growing through an epidermal cell. Any hypha reaching the surface may serve to receive spermatia and initiate the sporophytic (diploid) generation.

to the species that possess them in that they attract flies and other insects and thus insure that the function of the pycnia is fulfilled.

It has long been remarked that, in those rust fungi which possess them, the pycnia are the first spore-producing organs to appear. Since they play such an important part in changing the haplophase into the diplophase and in inducing the formation of aecia, their appearance on the mycelium in advance of the aecia can be readily understood: pycnia precede aecia because by their action aecia are formed.

Under natural conditions, there are at least two ways in which pustules of monosporidial origin may change from the haploid to the diploid condition: (1) by a (+) sporidium and a (−) sporidium settling on a leaf close together so that they form pustules which coalesce in such a way that the (+) mycelium and the (−) mycelium come into contact directly; and (2) by means of flies and other insects which carry the pycniospore-containing nectar of a (+) pustule to the pycnia of a (−) pustule or, conversely, carry the pycniospore-containing nectar of (−) pustule to the pycnia of a (+) pustule.

Whether or not a spontaneous change from the haploid to the diploid condition occurs in the mycelia must be further investigated. A phenomenon of this kind occurs in certain Hymenomycetes, but, in *Puccinia graminis* and *P. helianthi,* the apparently spontaneous change may be tentatively attributed to the fortuitous intermixing of nectar.

The crossing of two physiologic forms of *Puccinia graminis, P. helianthi,* or of any other heterothallic rust species which behaves like *P. graminis* and *P. helianthi,* may then take place in two ways: (1) by the union, within the tissue of one and the same host plant, of the (+) mycelium of a pustule belonging to one form with the (−) mycelium of a pustule belonging to a different form, or *vice versa;* and (2) by the application of the pycniospore-containing nectar of a (+) pustule of one physiologic form to the pycnia of a (−) pustule of another physiologic form, or by the application of the pycniospore-containing nectar of a (−) pustule of one physiologic form to the pycnia of a (+) pustule of another physiologic form. The second method is much more simple than the first, and it was used by all those investigators who have recently succeeded in crossing physiologic forms of rust.

SUMMARY

(1) A study was made of the sexual behavior of two rusts, *Puccinia graminis and P. helianthi.*

(2) An apparatus is described by which single sporidia can be picked off their sterigmata.

(3) Two methods of inoculation are described which make it possible to obtain monosporidial and bisporidial pustules.

(4) Pycnia developed on the upper side of each and every pustule of *P. graminis* and *P. helianthi,* and to a lesser extent on the lower side, being most abundant there in the peripheral regions of the older pustules.

(5) The pycnia of each and every pustule of *P. graminis* and *P. helianthi* exuded nectar containing numerous pycniospores.

(6) Both *P. graminis* and *P. helianthi* are heterothallic. Some evidence was secured which indicates that *P. coronata, P. Pringsheimiana* and a *Gymnosporangium* sp. (*corniculans*?) are also heterothallic.

(7) A large majority of the monosporidial pustules of *P. graminis* and *P. helianthi* failed to produce aecia.

(8) A small minority of the monosporidial pustules of *P. graminis* and of *P. helianthi* produced aecia at some time during the course of their development, possibly owing to a fortuitous mixing of their nectar by handling or through the agency of insects.

(9) Approximately half of the compound (bisporidial) pustules of *P. graminis* and of *P. helianthi* produced aecia, while the remainder produced none.

(10) The monosporidial pustules of *P. graminis* and of *P. helianthi* are of two sexes, (+) and (−). The (+) pustules are about equal in number to the (−) pustules. This fact indicates that, in all probability, the sporidia which are produced by the basidia and to which the pustules owe their origin are divisible into two groups, (+) and (−), which are about equal in number.

(11) Intermixing the nectar of monosporidial pustules of *P. graminis* or of *P. helianthi* induces the formation of aecia in the pustules so treated within from 5 to 6 days.

(12) Flies are active agents in carrying the nectar of one pustule to another and in thus affecting the transfer of pycniospores from (+) pustules to (−) pustules and of (−) pustules to (+) pustules.

(13) Nectar heated to 70° C. for 3 hours to kill the pycniospores does not induce the formation of aecia in the pustules to which it is applied.

(14) Some monosporidial pustules of *P. coronata, P. Pringsheimiana,* and a *Gymnosporangium* sp., found in nature, failed to produce aecia, thereby indicating that these species are heterothallic.

(15) Nectar of a (+) pustule applied to the pycnia in one part of a (−) pustule induces the production of aecia in that part to which it is applied, and rarely beyond that part.

(16) The origin of a new physiologic form of *P. graminis* is reported.

(17) Two methods are described by which a cross may be made between two physiological forms of a heterothallic rust which produces pycnia and aecia.

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INVESTIGATIONS ON THE BLACK ROOT OF STRAWBERRIES[1]

FORREST C. STRONG AND MIRIAM C. STRONG

INTRODUCTION

Among the cultivated crops the strawberry has long been considered one in which the disease hazard for the grower is small. Mildew, leaf spot, and a few fruit rots have at one time and another attracted the attention of growers. For the most part these diseases are of minor importance, and no control measures are practiced.

There is, however, a situation in strawberry culture which is a subject of concern to the growers. This consists in the failure of plants newly set out to become established and, often during the picking season, the dying of plants already established. In severe cases this latter condition may be so extensive as to render the plantation unprofitable. Losses in stand of plants in Michigan plantations have been observed to range from 5 per cent to as high as 60 per cent, in some cases, with consequent reduction in crop yields. A typical example is the case of a 1-year-old plantation located in the central part of the State where the grower set in new plants twice in an unsuccessful attempt to secure a full stand (Fig. 1). Many

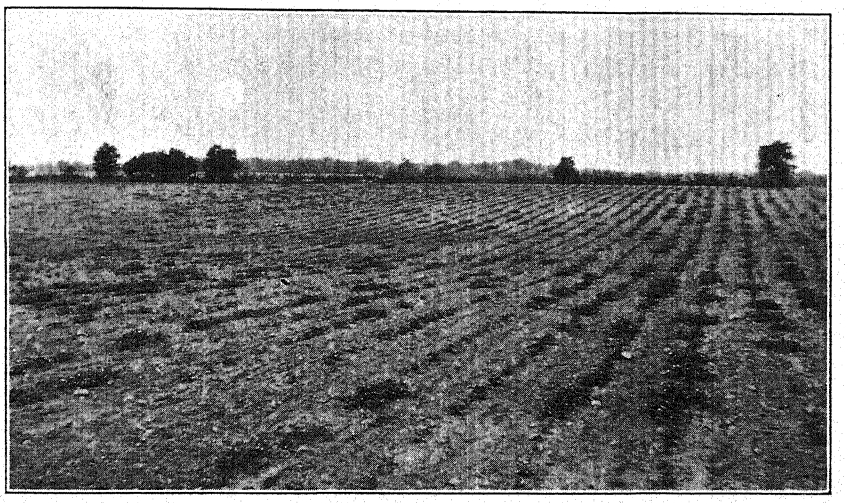


FIG. 1. A 20-acre plantation near Edmore, Michigan, showing effects of black root.

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other similar cases have been observed. Losses of plants in older plantations are particularly prevalent at the time of fruiting.

It is suggested that along with drought injury and winter killing, the disease that is the subject of this article is an important factor in the difficulties encountered in securing and maintaining a stand of strawberries. As the discussion proceeds, it will be seen that we are concerned with a root disease that, by its weakening effect on plants, renders them low in vitality and subject to injury by various factors, such as drought and frost. The conditions to be described will be seen to play an important rôle in the deterioration of strawberry plantations.

THE BLACK-ROOT DISEASE

A strawberry plant affected with black root has a characteristic appearance. The diseased plant shows low vitality, is usually stunted, and has a tendency to dry up at the onset of dry-weather conditions. Such a plant, on being dug up, is found to have a root system in which the roots are all black, corky in texture, and apparently dead. If a diseased plant, not yet in the wilting stage, be examined, there will be seen young white lateral roots pushing out from roots with blackened cortical tissue. On examination of such a root the diseased cortex will be found to peel off readily, leaving the vascular cylinder from which the secondary roots arose still more or less white and apparently functioning. It should be borne in mind that roots dying from age present much the same general appearance as that just described.

The examination of a younger plant affected with black root yields a more clean-cut picture of the disease. Even these younger plants show characteristic evidences of the disease by the production of smaller leaves and fewer main roots, together with the reduction in number of lateral roots. Young plants affected with black root lack vigor, as is manifested by their slow growth and reduced runner production. Some of the main roots of such plants will be rotted their entire length and brown to black. From few to many lesions are to be found on the main roots, and many or all of the lateral or secondary roots will either be rotted off or show distinct rotted areas (Fig. 2).

The lesions on the main roots vary in size from 0.5 to 5 cm. in length and usually encircle the root. At first they are reddish brown and later become black. These lesions, in their early stage, exhibit a brown, water-soaked appearance, but very soon become shriveled and shrunken. Very often the rotting involves all of the root tissue, affecting the stele or vascular cylinder, as well as the cortical tissue. Such roots break at the rotted portion. The lesions on the lateral roots resemble in miniature those on the main roots.

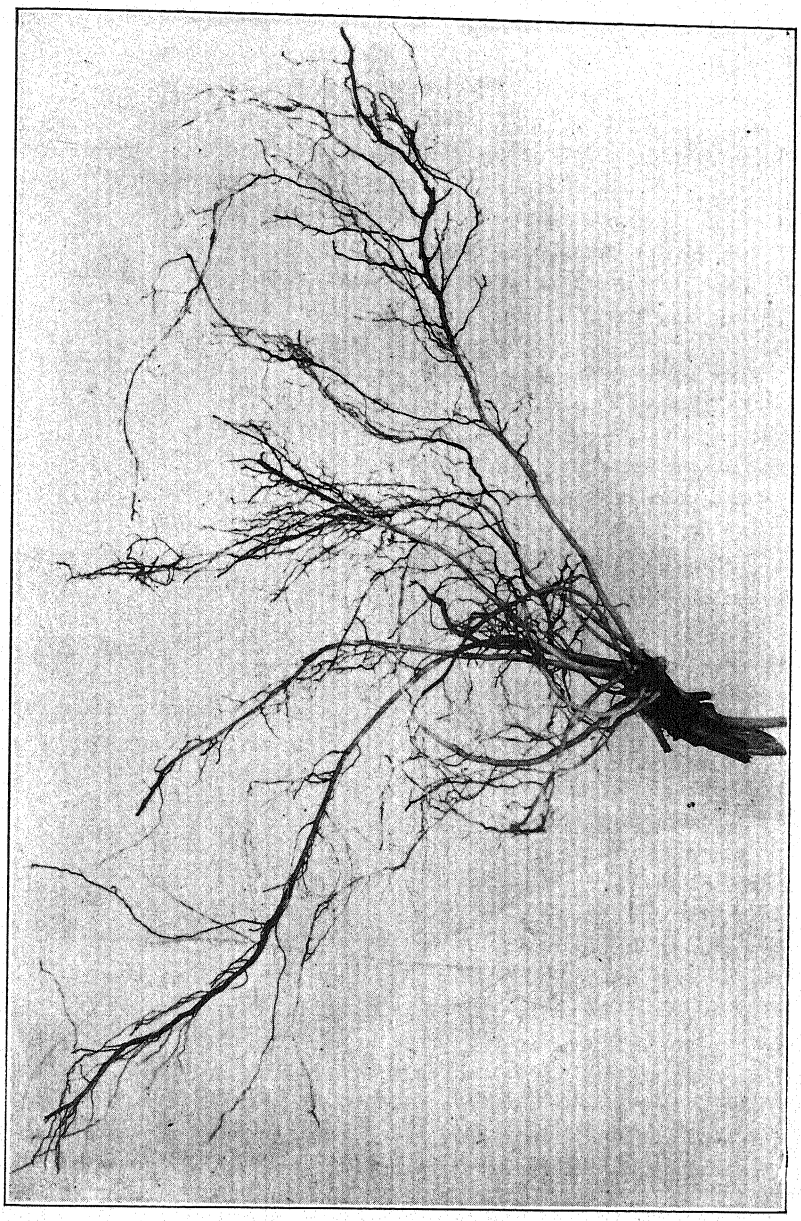


FIG. 2. Young plant showing natural infection.

It is probable that the attack upon the lateral roots is very important in producing the general effects of black root, since the root hairs through which water and plant-food materials enter the plant largely occur on these portions of the root system. The loss of these lateral roots then reduces the efficiency of the root system. This lack of a properly functioning root system is seen in plants affected with black root or root rot during dry periods of summer and especially during the picking season, if it is at all dry. On a hot day the leaves will wilt down and then recover during the night. If the dry weather continues, the plants will die, the berries shriveling and hanging green or partially ripened on the stem. The leaves frequently become purplish or bronzed, and the petioles turn red.

PREVIOUS WORK

Writers on strawberry culture have long referred to black root as a diseased condition of unknown cause. There is little to be gained by referring to these scattered notes, since none of the older writers made more than casual comment on the severity and probable cause.

What may be a typical case of black root was described by Clinton (5) under the heading "Leaf Scorch," but he stated that no fungus appeared to be affecting the roots. Fletcher (8) in 1917 describes "root rot or black root" of strawberry and states that the disease was prevalent in New York, Michigan, and Massachusetts in the years 1902 to 1908. "When the berries are about half grown the plants wilt and turn yellow; the roots are decayed. Most of this trouble is due to winter injury, but a bacterial disease is associated with it in some cases. Poor culture, lack of fertility, the crowding of plants in the row, insufficient mulching, and wet land are favorable for this trouble." Heald (10) in 1920 mentioned the "dying out" of strawberry beds in western Washington and ascribed the trouble to Rhizoctonia. The next year Smith and Horne (16), in California, described a rot of strawberries in which the cortex of the root decayed. No parasite was found and the rot was believed due to water-logging of the soil or sudden drying out of soil moisture. In the summer of 1923 the disease was reported from Mississippi by Neal (11), who wrote: "A root rot of strawberry has been discovered in many parts of the state the present season. The disease causes a distinct wilting of the plants, accompanied by pronounced discoloration of the roots and crown tissue. A species of Fusarium has been isolated from the affected crown, but at present it is not known if this fungus is parasitic or responsible for the death of the plants. This trouble is serious and is causing many growers of strawberries considerable alarm."

Investigations on black root date from this time and are not very extensive. In 1923 Berkeley and Jackson (3) reported the isolation of vari-

ous soil bacteria that they thought to be the causal organisms. Later, in February 1924, Berkeley (4) further reported black root present the second season and doing much damage. The soil bacteria isolated previously failed to cause infection when tested by inoculation into plants. A species of Fusarium was suspected and mycelium was found in sections of diseased roots. In 1924 Sherbakoff (15) reported black root as a disease of wide occurrence in the Southern States and also farther north. He stated that it is of considerable economic importance in certain sections. He isolated a fungus from the diseased roots, but was unable to identify it because it remained sterile in culture. Coons (6) also, in 1924, described black root of strawberries in Michigan and stated that the conditions of infection indicated Rhizoctonia as the causal organism. In 1926 Darrow (7) summed up the situation as follows: "There are several troubles known as black root which are caused by fungi or bacteria. No thorough studies of these troubles have ever been made, but their effect is to kill the roots and so shut off the intake of water and dissolved minerals." Again: "Little is known of the cause or control of crown or root diseases of strawberries. They are often very serious and may cause serious injury which is not recognized as due to disease. They may even be the most important of all strawberry diseases."

Late in 1926 Wardlaw (22) reported on the Lanarkshire disease of strawberries in Scotland. His description is somewhat similar to the black-root disease in this country. He isolated a Pythium and an unnamed member of the *Fungi Imperfecti* that, upon inoculation, produced typical symptoms of disease. In 1927 Ball, Mann, and Staniland (2), reporting the strawberry investigations at Long Ashton, England, describe "sudden wilt" of strawberry. Fungi were isolated, but no proof of pathogenicity was obtained. Although the condition of the root system is not mentioned, the general symptoms resemble those observed in black root. In 1928 Thomas (21), in discussing the killing of strawberry roots in New York State, concluded that the root killing may result from a number of causes and that fungi play a minor rôle. Alcock (1), in 1929, stated that the name "Lanarkshire disease" has been used to include several strawberry-root troubles. She described a root rot that she called the "red core root" of strawberry. This disease is, apparently, not the same as black root. In 1930 Plakidas (13) reported on the root rot of strawberries in Louisiana. Several unidentified species of Pythium were isolated, one of which proved to be very pathogenic when used to inoculate healthy plants.

OCCURRENCE OF THE DISEASE

The black root of strawberries is widely distributed in the United States and Canada. The disease occurs in Mississippi, Louisiana, Tennes-

see, Alabama, New York, Ontario, California, and Washington. On the trip made by the 5th Annual Meeting of The American Phytopathological Society (9) in 1923, through western New York and Ontario, Canada, it was observed at various places.

Collections in Michigan have shown the disease widely distributed in the strawberry-growing districts of the State. It is evident that this disease is extremely wide-spread and coincident with the culture of strawberries. Since strawberry plantations are established with nursery plants, and new varieties are constantly being introduced by nurseries, one might suspect that we are dealing with a disease of strawberries distributed perhaps on nursery stock and thus becoming introduced and established in the fields.

Opposing this view is the fact that plants of native species have been found either killed out entirely or showing marked evidences of the disease by the presence of lesions and rotted roots. These wild plants have been found in many places in the State, and many cases have been observed in locations where strawberries have never been grown as a crop. It will be remembered here that wild-strawberry patches arise from seedling plants, the seeds having been carried by birds. This situation, together with the wide-spread distribution of the disease, strongly indicates that the disease occurs more or less irrespective of soil types, and the cause is to be sought among the local soil organisms.

THE NATURE OF BLACK ROOT

As has been said, examinations of the roots of plants affected with black root showed rotted roots and browned and blackened areas of varying size. (Fig. 3, A, B, and C). These lesions indicate a parasitic organism as the cause.

It has been pointed out that the situation was confused by drought and winter injury. In order to determine whether we were concerned with a disease caused by some parasitic organisms and not by soil or climatic factors, a series of preliminary experiments were performed.

Dead and dying plants typical of the black-root condition were dug up and their root systems chopped into small pieces. This mass of débris was placed in pots so that strawberry plants could be grown with new roots passing through this material. A series of pots were filled two-thirds full of clean sand, a 1-inch layer of débris added, and the pot then filled with sand. As a control, pots of clean sand with no black-root inoculum were employed. Also, pots containing a layer of débris, previously sterilized by autoclaving at 15 lbs. pressure for 30 minutes, were used as additional controls. Young runner plants with root tips just showing were set into these pots. Frequent waterings were made to keep the sand and débris

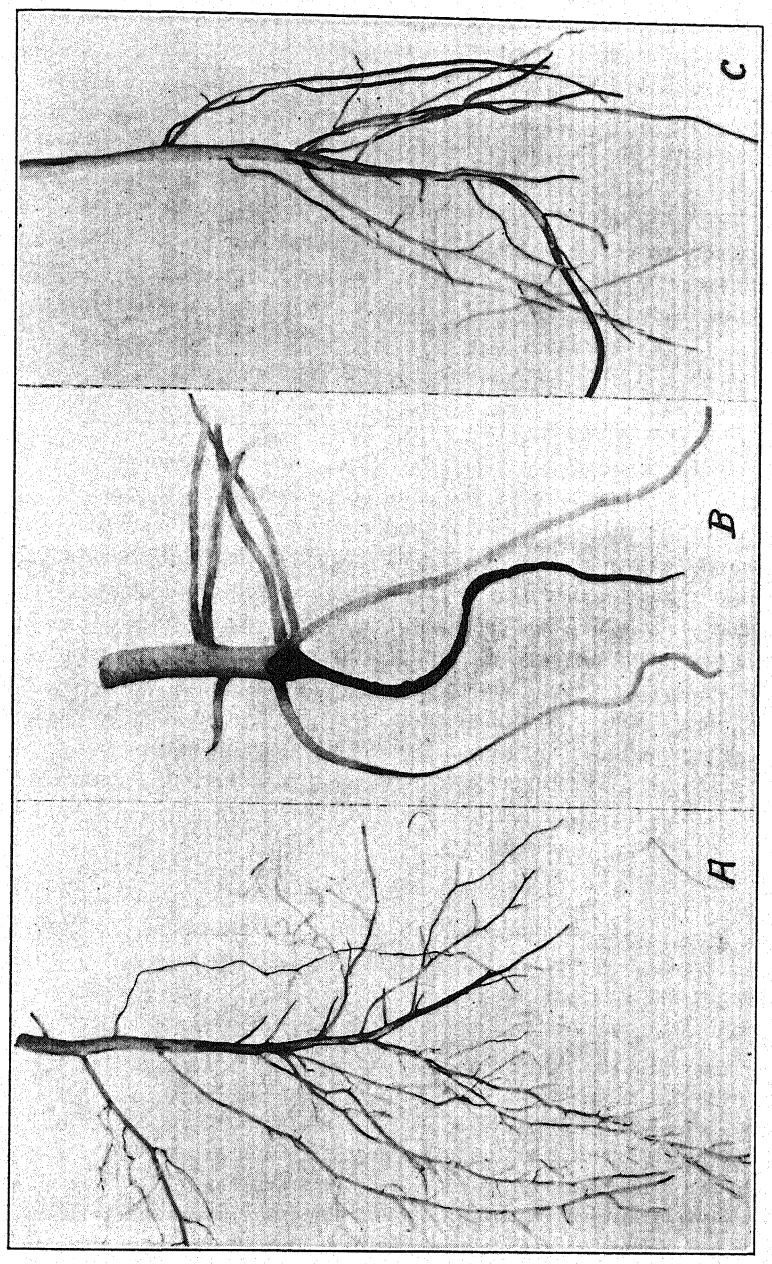


FIG. 3. A. Typical lesions on main root with many lateral roots infected. B. The rotting of a main root from the tip (×3). C. Infected root showing characteristic absence of fibrous roots and many lesions with healthy root tissue adjoining.

inoculum moist. Examinations and readings of the condition of the roots were made in about 14 days. The result of the test as shown in table 1 was

TABLE 1.—*Results of inoculation of clean runner plants with strawberry roots naturally infected with black-root disease*

	Total no. plants	No. plants infected	Infection per cent	Total no. lesions	Average no. lesions per plant
Inoculated: clean sand plus black-root material	32	29	90	104	3.2
Control: clean sand	7	0	0	0	0
Control: clean sand plus autoclaved black-root material	13	1	7.6	3	0.2

conclusive. In this experiment typical black root appeared on 29 out of 32 inoculated plants, while, in the sand controls, all the plants remained healthy, as evidenced by their white roots. The 13 plants inoculated with sterilized débris showed 12 plants healthy, while 1 plant showed 3 lesions. A plausible explanation of this is that it may have been natural infection due to presence in a diseased field and in an open pot. The great number of lesions appearing on the roots of inoculated plants as compared with the clean plants in the sand controls, as well as the plants inoculated with sterilized débris, indicates quite clearly that the disease is infectious and transmissible from one plant to another and parasitic in nature rather than attributable to some soil factor or to winter injury. Examination with the microscope of sections made from diseased roots gave abundant evidence of septate hyphae in the root tissues.

ISOLATION OF THE CAUSAL ORGANISMS

Many plantings were made of bits of blackened roots in Petri dishes containing nutrient medium. Material for use in making such plantings was procured partly from strawberry plants sent in to the Botanical Section of the Experiment Station for disease diagnosis and partly from plants collected by the writers and others of the botanical staff in various parts of Michigan where strawberries are grown extensively.

Prune agar was commonly used as the medium for isolation purposes since its slightly acid reaction discourages bacterial growth. Potato-dextrose agar, corn-meal agar, and strawberry-root agar also were used. The technique of making these plantings consisted in washing the roots carefully to remove the dirt. They were then dried of excess moisture on sterile paper toweling and split lengthwise, the splitting being initiated merely by sterile needles or forceps. Plantings were made from the in-

terior tissues now exposed and that had not been touched by anything in order that no contamination might be introduced. Washing the roots with disinfectants was unsatisfactory, since no growth resulted from plantings made from materials so prepared.

The range of material employed and the number of plantings made are believed by the writers to be extensive enough to afford a fair sampling of black-root material as it occurs in Michigan and to indicate the organisms chiefly involved. The isolations from diseased tissue were extended over a period of 6 years and involved the making of more than 3,500 isolations from diseased plants collected in various parts of the State. The fungi appearing most often in these plantings were: a species of Coniothyrium and a fungus tentatively identified as a species of Gloeosporium and later as a species of Patellina (20) but that we now believe to be identical with the *Patellina fragariae* of Stevens and Peterson (17) and *Hainesia lythri* (Desm.) v. Höhn. shown by Shear and Dodge (14) to be one of the imperfect stages of *Pezizella lythri* (Desm.) Shear and Dodge. Other organisms appearing occasionally were: a brown Fusarium, a red Fusarium, *Pythium* sp., *Acrostalagmus* sp., as well as various species of Mucor and Penicillium.

The Hainesia was isolated also several times from sporodochia on the lower leaves and from the inner tissue of black, shriveled peduncles and petioles of strawberry plants showing heavy natural black-root infection. These plants were badly wilted, and finally died with green berries hanging on the black and shriveled peduncles. The lower leaves were dark brown with abundant cream-color sporodochia, and the root system presented the typical appearance. The roots were all black and rotted their entire length, so that many of them broke off when the plants were removed from the soil.

PATHOGENICITY TESTS OF SUSPECTED ORGANISMS

Following the isolation of the *Coniothyrium* sp. and *Hainesia lythri* in plantings from diseased tissue, these organisms were secured in pure cultures and tested by repeated transfers. Several series of inoculation experiments were set up to test the pathogenicity of these isolations.

Inoculation of Strawberry Seedlings.—The *Coniothyrium* sp. and *Hainesia lythri* were used to inoculate strawberry seedlings grown in deep culture dishes on sterile sand, kept moist with sterile Knop's nutrient solution. Both organisms were able to infect strawberry seedlings. The result of several series of seedling inoculations are arranged in summarized form in table 2. Although several control plants died during the course of the experiment, these plants showed no fungi, externally nor internally. This is thought to be the natural death of less vigorous plants due to lack of nutrition.

TABLE 2.—*Summary of results of inoculations of strawberry seedlings with Coniothyrium sp. and Hainesia lythri*

Inoculated with *Hainesia lythri*			Inoculated with *Coniothyrium sp.*			Control		
No. of plants	Time	Plants infected	No. of plants	Time	Plants infected	No. of plants	Time	Plants dead
			12	13 days	12	46	13 days	0
21	11 days	20	24	13 days	21	42	11 days	2[a]
195	21 days	184	383	21 days	363	111	21 days	16[a]

[a] These plants showed no fungi, externally nor internally. This is thought to be the natural death of less vigorous plants due to lack of nutrition.

Some time after the infection of those plants inoculated with the *Coniothyrium* sp., pycnidia appeared on the roots, stems, and seed leaves. The pycnidia were submerged in the tissue of the host.

FIELD TESTS OF PATHOGENICITY OF CONIOTHYRIUM SP. AND HAINESIA LYTHRI FOR STRAWBERRY ROOTS

The next series of inoculations were undertaken in the field, where runner plants were set in pots filled with clean sand, the purpose being to secure the natural conditions of plant growth and yet avoid as much as possible the contamination from the soil in which the mother plants were growing. In this way it was sought to demonstrate that the two organisms shown to be capable of producing black root under laboratory and greenhouse conditions could produce the field form of black root. For the most part, Premier and Senator Dunlap were the varieties used in these tests. The runner plants remained attached to the mother plants throughout the entire period of the experiments. The former were selected before any roots had started, were treated with a 4 per cent chlorazene solution for 5 minutes, and set so that all root production was made in pots of clean sand. The pots were watered often enough to keep the sand constantly moist.

The inocula consisted of pure-culture material of the *Coniothyrium* sp. and *Hainesia lythri* grown on various media, such as prune juice, potato-dextrose agar, and corn-meal mush. In the first of this series of inoculations, spore suspensions were used. Later, mycelial masses were used in small quantities, and it was found that the inoculations giving the highest

percentage of infection in the shortest time were those in which large pieces of mycelial mats were used. In some experiments the inoculum was placed in the sand, and the roots were allowed to strike and grow down through the inoculum. In other experiments the roots were allowed to strike in the clean sand, and the inoculum was added when the roots were at least 1 inch long. The plants were then removed from the sand and reset. The control plants were handled in a similar manner except for inoculation.

The disease developed in 10 to 21 days. At intervals of about 2 and 4 weeks the plants were removed from the pots and examined. They were then either carefully reset so that later readings could be made or were brought to the laboratory and photographed, and reisolations were made from the lesions.

During the course of this investigation, many such tests were made, all of them giving conclusive evidence of the pathogenicity of the two organisms. The data of these tests, being too extensive to be given in full, are summarized in table 3.

The type of experiment possible under field conditions, if one desires to work with plants as nearly normal as possible, is one in which a medium free from infectious material is used for rooting the runners. The possibility of chance introduction of organisms on the runner plant, from dust and rain splashings, is considerable, and proper conclusions from experiments with soil organisms, such as those considered here, must be based on the relative amounts of infection in inoculated plants and in the similarly handled controls.

It will be noted from a study of table 3 that, in general, the control plants show a small amount of infection that is to be regarded as casual infection, practically unavoidable in field work of this nature. Comparison of the infection in the control plants with the far greater infection found in the inoculated plants, as shown in columns 6, 11, and 16, table 3, the writers believe to be conclusive evidence of the pathogenic nature of the two organisms under consideration.

The data given in columns 3, 4, 5, 8, 9, 10, 13, 14, and 15 of table 3 are from actual records made in the field. The figures in columns 6 and 11 are obtained by dividing the total number of lesions by the total number of plants inoculated. The figures in column 16 are obtained in like manner for the control series of the experiment. The 7th and 12th columns show the relative percentage of infection in the inoculated plants of a series that is properly to be attributed to the inoculation. These percentages were obtained in each series by finding the difference between the average number of lesions per plant in the control and the average number of lesions per plant in the particular series of inoculated plants considered. This

TABLE 3.—*Summary of results of inoculations of clean runner plants, under field conditions, with Coniothyrium sp. and Hainesia lythri*

		Coniothyrium sp.					*Hainesia lythri*					Corresponding controls			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1
Date of inoculation	No. days duration	Total no. plants	No. plants infected	Total no. lesions	Av. no. lesions per inoculated plant	Infection due to inoculation per cent	Total no. plants	No. plants infected	Total no. lesions	Av. no. lesions per inoculated plant	Infection due to inoculation per cent	Total no. plants	No. plants infected	Total no. lesions	Av. lesi p con pl
1926															
July 10A	10	24	11	26	1.08	70	14	5	22	1.57	80	25	6	8	.3
"	27	24	20	127	5.29	87	14	12	104	7.42	91	[a]6	3	4	.6
July 10B	27	24	13	65	2.70	75	40	27	104	2.60	75	[a]6	3	4	.6
July 21	14	31	5	13	.42	88	54	5	11	.20	75	[b]39	2	2	.0
"	37	31	31	560	18.06	85	54	43	350	6.48	59	47	35	126	2.6
July 22	11	33	32	376	11.39	99	25	13	72	2.88	98	[b]39	2	2	.0
August 2	11	10	10	143	14.30	100	13	12	94	7.22	100	[c]9	0	0	
"	29						13	13	113	8.69	72	[d]9	7	22	2.4
August 4A	8	18	14	145	8.05	100	19	16	117	6.15	100	[c]9	0	0	
August 4B	27						6	6	75	12.50	80	[d]9	7	22	2.4
August 17	14						16	16	218	13.62	97	29	5	11	.3
August 24	14						18	16	142	7.88	71	27	12	62	2.2
October 2A	26	6	6	100	16.66	92	9	9	126	14.0	90	[e]23	10	32	1.3
October 2B	26	10	10	117	11.70	88	10	10	71	7.10	80	[e]23	10	32	1.3
Aug. 1929	22						85	39	123	1.44	88	41	3	7	.1
Aug. 1930	30	95	83	385	4.05	84	100	93	540	5.40	88	104	26	69	.6
		Total 251	Total 219	Total 2018	Average 8.04	Average 88.2	Total 409	Total 313	Total 2155	Average 5.27	Average 81.9	Total 359	Total 109	Total 343	Ave 0.9

[a], [b], [c], [d], [e] Repetition, not separate series.
The two records in each brace represent readings, at different dates, on the same experiment.

difference, divided by the average lesions per plant for this particular series of inoculations, gives the percentage of infection that may reasonably be attributed to the inoculation. Expression of the results in this manner avoids the distortion that would arise if the percentage of infection were based on the number of plants showing lesions on the roots. A control plant showing one lesion would be given equal weight with an inoculated plant that might have 10 to 20 or more lesions.

Sterile sand was used in some of the inoculation experiments, but the amount of infection appearing in the control plants was not decreased by this procedure, indicating that the infection was due to infectious material, blown, or splashed by water, into the sand from outside the pot. Isolations made from lesions on control plants showed a large percentage of the two organisms under consideration.

The lesions and rotted roots found and recorded on the inoculated plants were identical in appearance with the lesions and rotted roots found on plants growing in cultivated strawberry fields and on wild plants. These lesions at first are brown, shriveled areas usually encircling the root and having white healthy root tissue adjoining. These lesions were observed to lengthen until an entire root was involved. The particular organism used in the inoculation was recovered many times from such lesions. Typical infection produced by inoculation is illustrated in figure 4, A and C, while B shows a typical control plant.

INOCULATION OF FRUIT

Ripe berries were carefully washed and placed in sterile moist chambers. They were then inoculated with spores or bits of mycelium, the epidermis of the berry being punctured. The Hainesia caused a rapid leathery rot of the fruit with the production of abundant fruiting bodies (Fig. 5, A), the spore heaps being of a cream or strawberry color. This closely resembles the rot described by Stevens and Peterson (17) for their *Patellina fragariae* and the rot caused by *Hainesia lythri* described by Shear and Dodge (14).

The *Coniothyrium* sp. caused a soft rot of the fruit, but no fruiting bodies were produced.

ORGANISMS INVOLVED

The organisms, shown by the writers to have been concerned in the black-root disease of strawberries, are the imperfect stages of two Ascomycetes. The Coniothyrium species is morphologically very similar to *Coniothyrium fuckelii* Sacc., shown by Stewart (18) to be the conidial stage of *Leptosphaeria coniothyrium* (Fckl.) Sacc. The spore size is within the limits given by various authorities for *C. fuckelii* on other

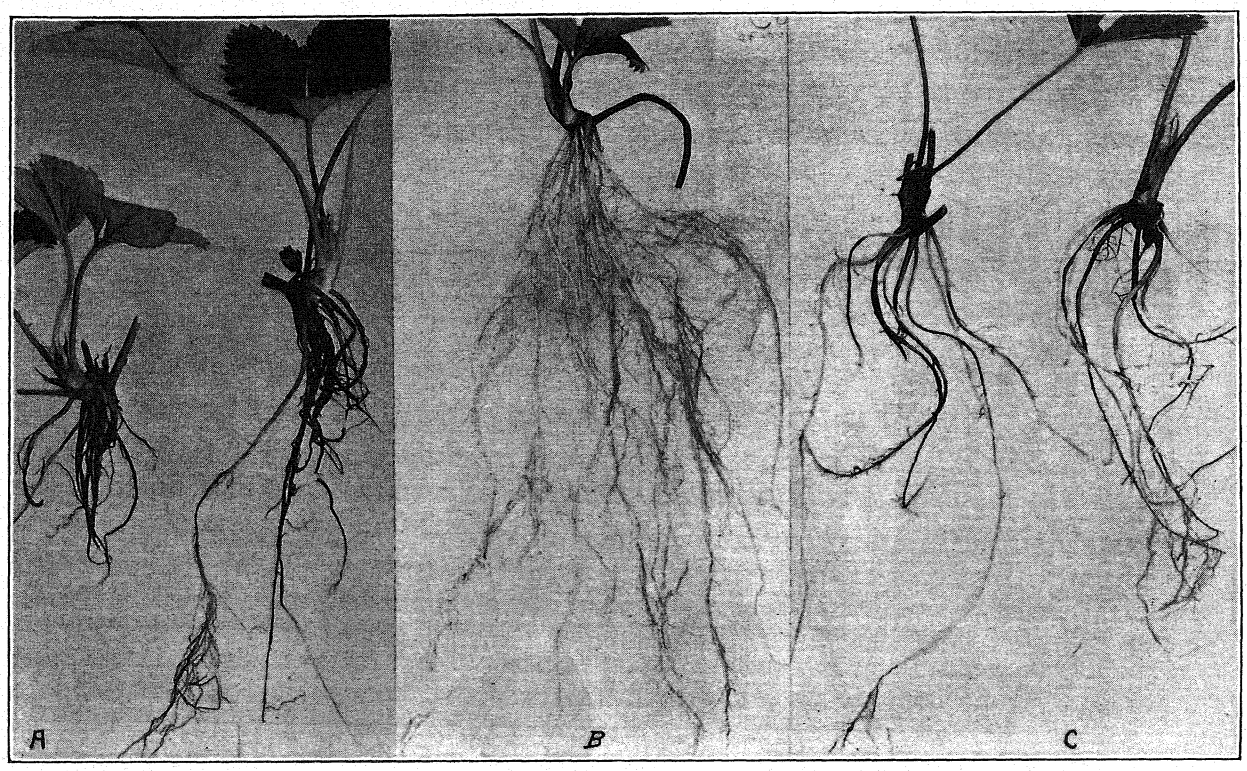


FIG. 4. A. Plants infected by inoculation with *Coniothyrium fuckelii*. The right side of the larger plant is so rotted that many of the roots are broken off. B. Typical control plant. C. Plants infected by inoculation with *Hainesia lythri*.

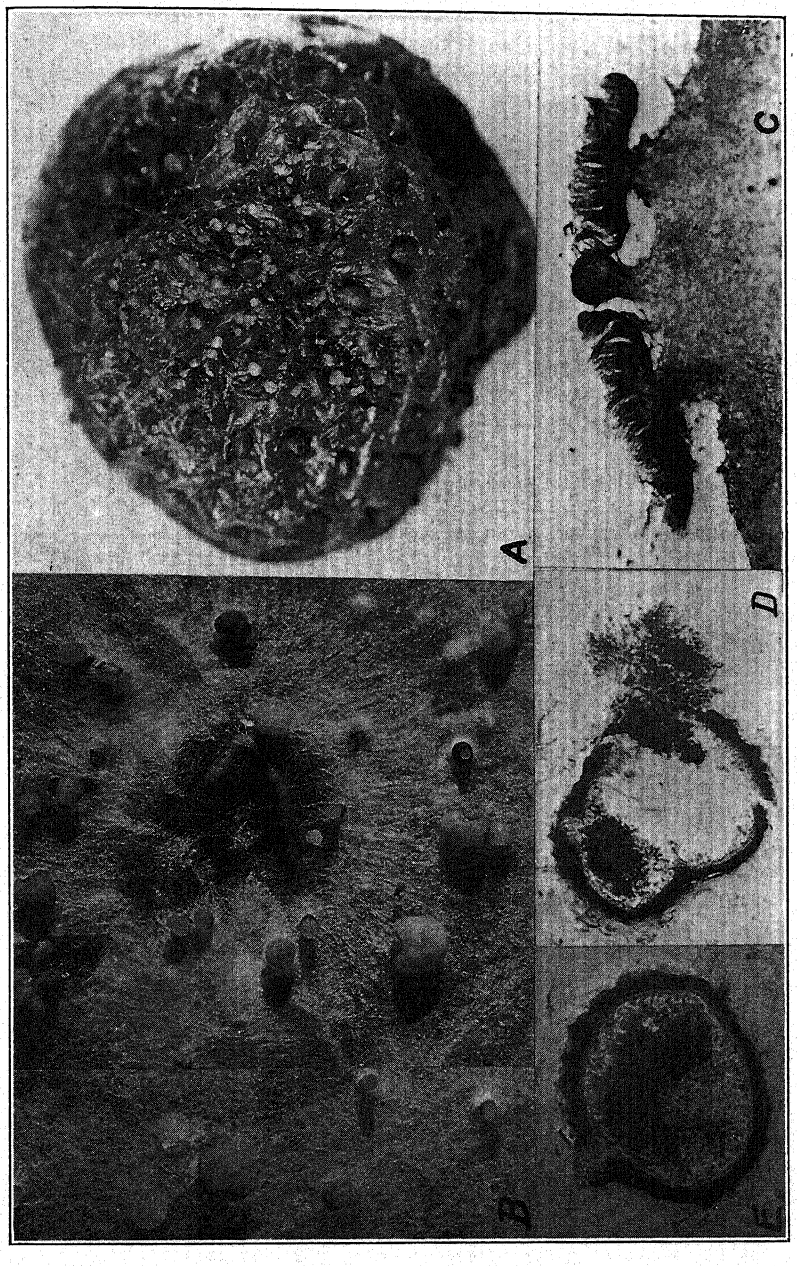


FIG. 5. A. Sporodochia of *Hainesia lythri* on strawberry (×3). B. Several types of sporodochia of *Hainesia lythri* on potato-dextrose agar (×10). C. Vertical section of sporodochia of *Hainesia lythri* showing stipe (×80). D. Section of pycnidia of *Coniothyrium fuckelii* showing spores (×160). E. Section of pycnidium of *Coniothyrium fuckelii* showing ostiole (×200).

Rosaceous hosts. The strawberry black-root Coniothyrium apparently is not the same as *C. fragariae* Oud., which is described as having considerably larger spores (11.6 × 9.3 μ). Cultural and histological studies indicate that the fungus, first considered a *Gleosporium* sp., is identical with the *Patellina fragariae* of Stevens and Peterson (17) and *Hainesia lythri* (Desm.), v. Höhn., shown by Shear and Dodge (14) to be the same fungus and one of the conidial stages of *Pezizella lythri* (Desm.) Shear and Dodge. The following descriptions record the salient characteristics of these organisms.

Coniothyrium stage of *Leptosphaeria coniothyrium* (Fckl.) Sacc.

The pycnidia are ovoid to pyriform with a definite round ostiole, without prominent lips; black when filled with spores, but appearing dark brown after the spores are discharged. Walls thin, almost membranaceous. Pycnidia 160 μ to 200 μ in height and 140 μ to 170 μ in diameter. (Fig. 5, D and E).

Spores exuding in an inky black mucilaginous mass that accumulates at the apex of the pycnidium. Seen singly the spores are fuliginous, unicellular, short, ellipsoid to ovoid, round at both ends and usually 2-guttulate. Size 3.8–4.8 μ × 2.7–3.7 μ, average 4.2 × 3.1 μ.

Hainesia stage of *Pezizella lythri* (Desm.) Shear & Dodge.

The fruiting bodies of this fungus vary in shape from the sporodochium typical of Tubercularia to a cup-shape, stiped body resembling an apothecium. In some cases the cup is nearly closed so that the exuding spore mass forms in a spore horn suggesting the true pycnidial type of fruiting body. Sporodochia on the tissues of the strawberry plant, 70–130 μ in diameter. In culture, often much larger, reaching a size of 700 μ (Fig. 5, B). The wall of the cup is 5 or 6 cells thick, dark brown especially in the basal portion. Sometimes the cups are nearly sessile, although many of them have very pronounced stipes (Fig. 5, C). Spores are produced singly on simple or sparsely branched conidiophores. Early conidiophores are short, but, as the mass of slime-embedded spores increases, the later conidiophores elongate to penetrate this mass. Conidiophores hyaline, slender 20–60 μ long (Fig. 6, A). Spores hyaline, unicellular, slightly curved, oblong with pointed ends, 6.6–9.9 μ × 1.8–2.2 μ (Fig. 6, B). In mass the spores are cream-color when young to brown with age. On ripe strawberry fruits the spore masses are often strawberry-color.

The pycnidial and perfect stages described by Shear and Dodge (14) have never been seen by the writers, either in culture or on strawberry plants, but the organism in question resembles very closely the description and figures they give for the conidial stage of *Pezizella lythri*.



FIG. 6. A. Camera-lucida drawings of conidiophores of *Hainesia lythri* (×700).
B. Drawings of mature spores of *Hainesia lythri* (×700).

ROOT-TREATMENT EXPERIMENT

Along with the work of isolating the causal organisms and determining their pathogenicity, attempts were made to find a root treatment that would be effective in checking or controlling this disease. Mercuric chloride, formaldehyde, and two organic-mercury compounds, Semesan and Uspulun, were used. These compounds were dissolved in water in various concentrations, and the plants were plunged up to the crown in the solutions for periods of 30 minutes before setting in the experimental field. Each treatment appeared six different times in the field, nine rows apart. Every third row was set with nontreated plants as a control. Results do not warrant further details of the experiment, since lesions and rotted roots were found to be as abundant on the treated plants as on the nontreated. The control plants, for the most part, made more vigorous growth than the treated plants.

DISCUSSION

Evidence is presented of the parasitic nature of the black-root disease of strawberry, and the proof is given for assigning the disease to two organisms, *Coniothyrium fuckelii* and *Hainesia lythri*. The rôle that climatic factors play in augmenting the effects inaugurated by these organisms has been mentioned. Inoculation experiments have shown that the two fungi described in this paper are capable of infecting healthy, uninjured strawberry roots, as well as roots that had been injured by removal from, and resetting in, the sand in which they were grown. Thus the inoculation experiments are comparable to the conditions of infection both of uninjured plants, well established in the soil, and runner plants that have had the roots injured by transferring to new plantations.

As has been shown, black root is to be found generally distributed in strawberry plantations in Michigan and elsewhere. The two organisms whose pathogenicity has been demonstrated have been isolated from strawberry roots gathered from many more or less distant places. The Hainesia stage of *Pezizella lythri* has also been isolated from rotted petioles and peduncles and from sporodochia on the lower leaves of plants, indicating the possibilities of rapid dissemination of this fungus in strawberry plantations during rainy weather. The similarity of the lesions found on cultivated and wild strawberries makes it seem possible that the wild plants also are affected with a black-root disease of the same nature as that of the cultivated plants. This suggests that these fungi may be soil organisms of wide distribution. The fact that in this study two organisms have been found, each capable of producing typical black-root conditions, together with the fact that the literature of the disease shows other organisms assignable as pathogens, makes it seem probable that the black-root condition in strawberries may be the result of attack by a number of pathogens working singly or together. Both of the fungi described in this article are capable of producing a rot of the strawberry fruit.

The two organisms isolated in this work are known to be widely distributed and to have a wide host range. Shear and Dodge (14) list Rubus, Oenothera, Acer, Epilobium, Cornus, Smilax, Rhus, and Fragaria as hosts of the Hainesia. *Coniothyrium fuckelii* has been shown to be actively parasitic on other Rosaceous species. O'Gara (12) showed that this fungus causes a canker of rose and apple and a fruit rot of apple. Stewart and Eustace (19) proved *C. fuckelii* to be the cause of cane blight of raspberries.

Although some work has been done on the control phase, the treatment of roots has not been successful in checking the disease. Since the organisms causing this disease are wide-spread, root treatment will be useless. Whether these organisms and others that may cause the disease were originally spread by diseased plants that developed in nurseries cannot be said. This is probably not the case, but the growers of plants for sale should furnish as strong, vigorous, and healthy plants as possible. The grower who sets plantations for the fruit crop must select only this kind of plants for setting. Where root rot is severe, it is advisable to follow a one-crop rotation. Strawberries should be planted in soil where strawberries have not been grown for some years or, if possible, where strawberries have never been grown. The leading nurserymen practice this method to obtain as vigorous and as healthy plants as possible. The ultimate control is, as with so many plant diseases, probably to be sought in the selection or breeding of disease-resistant plants.

SUMMARY

1. Black root of strawberry was found from reports in the literature and collections made by the writers to be a wide-spread disease of cultivated and wild strawberries. Its etiology was not well-known.

2. By inoculation with strawberry débris, the infectious nature of the disease was determined and microscopic examination showed constant association of fungus hypae in the lesions.

3. The Coniothyrium stage of *Leptosphaeria coniothyrium* (Fckl.) Sacc. and the Hainesia stage of *Pezizella lythri* (Desm.) Shear and Dodge have been isolated many times from typically diseased roots and their pathogenicity to plants grown under controlled conditions has been proved.

4. In several series of field-inoculation tests, using runner plants rooted in clean or sterile sand, the typical disease has been produced by each organism, thus leaving no doubt of the etiological relations of these two forms.

5. Technical descriptions of these two species of organisms are given.

6. Treatment of strawberry plants with standard disinfectants before setting did not control black root.

7. General control measures, such as would make for strong, vigorous plant growth and rotation of crops, are recommended. Ultimate control of this disease will doubtless depend on selection of resistant strains.

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FURTHER NOTES ON PLANT DISEASES IN PERU[1]

E. V. ABBOTT[2]

Since the publication of an earlier paper (1) on the diseases of economic plants in Peru several diseases hitherto unreported have come to attention, and information regarding the distribution and importance of others has been accumulated, much of which is of general interest. It appears desirable, therefore, to make a further report on the plant diseases of the country.

SUGAR CANE

1. Sugar-cane mosaic in the Carabayllo Valley has been found to be more widespread than at first reported. Few fields are entirely free of the disease and many of them, particularly the old rattoons, show 90 to 100 per cent infection. It was at first thought that eradication offered the most effective means of control, but, in view of the general distribution of the disease on the plantations of this valley and the indifference toward it on the part of most of the planters, eradication appears to be impractical. Control measures should be concentrated rather on the introduction and testing of more resistant varieties in case mosaic should cause the failure of Otaheite or Bourbon, of which there is little indication at present, even where the disease has been present for 8 to 10 years. Field trials of resistant varieties, including several of the P. O. J.'s, have been initiated.

Although surveys have been made in every important cane-growing valley in Peru for the purpose of determining the presence of mosaic, the disease has not been found outside of the Rimac area. A strict quarantine has been placed on the exportation of seed cane from this region to other parts of the country.

2. The mechanical twisted top is occasionally observed affecting Otaheite, but it is of no importance.

COTTON

1. Fusarium wilt is rapidly becoming more serious in the valleys where it occurs, particularly in the Rimac and Carabayllo, and is causing the abandonment of cotton culture on several plantations. Each year the disease appears in fields where it had not been observed before, the spread apparently being due to inoculum carried by irrigation water.

[1] Contribution from the Department of Plant Pathology, Estacion Experimental Agricola, Lima, Peru.

[2] The writer is indebted to Dr. J. C. Arthur for the identification of *Puccinia glumarum, P. Pittieriana,* and *Aecidium cantensis;* to Dr. C. L. Shear for *Phyllachora maydis* and *Phoma zeicola;* to Dr. C. D. Sherbakoff for *Fusarium martii-minus* and *F. solani;* and to Dr. Charles Chupp for *Cercospora zebrina.*

Certain varieties of American Upland cotton, developed in the United States for their resistance to wilt, have failed to show promise when planted in wilt-infested soil in Peru. In experiments conducted at Lima during the seasons of 1929 and 1930, Rowden 40, Rowden 2119, Super Seven, Dixie Triumph, and Express all proved to be more susceptible to wilt than the native Tanguis and had the additional disadvantage that, when planted in wilt-free soil, their production was much less than that of the native variety.

These varieties were planted on October 10, 1929, in soil known to be heavily infested with the wilt fungus. Symptoms of wilt began to appear late in December and the first counts were made on January 1, 1930, just prior to the blooming period. The average percentages of wilted plants at different dates during the season are shown in the following table:

TABLE 1.—*Percentage of plants showing infection by Fusarium vasinfectum at different dates during the 1930 season*

Variety	Jan. 1	Feb. 1	Mar. 1	Apr. 1	June 1
Tangius strain 562	3.2	8.8	36.4	41.7	100
" " 762	3.5	13.5	31.3	67.8	100
" " 2072	2.1	8.8	25.1	32.4	100
Dixie Triumph	3.2	17.7	22.4	100.0	Dead
Rowden 40	2.0	15.0	30.0	100.0	"
" 2119	2.3	15.0	28.7	100.0	"
Express	1.3	19.5	27.0	100.0	"
Super Seven	3.0	12.6	22.7	100.0	"

It will be noted that, although during the early part of the season the Tanguis strains showed about the same degree of infection as the American varieties, Tanguis stood up much better as the plants approached maturity. By June 1 all of the American varieties had been killed by wilt and produced no crop of any consequence, while the Tanguis strains continued to mature a large percentage of the bolls that had set, in spite of the fact that all of the plants showed indications of wilt infection. There was, however, considerable shedding in the Tanguis and the yield was materially reduced.

It is customary in most sections of Peru to grow from one to three rattoon crops of cotton from a single planting, and in the rattoons the greater wilt resistance of Tanguis is more marked than in the plant crop. Although the number of plants of this variety emerging the second season may be reduced from 10 to 50 per cent by wilt, the American varieties fail entirely, having been killed by the disease at the end of the first season. Notwithstanding this greater resistance, however, the yields of the Tanguis rattoon crops are materially reduced, and it is their failure that has forced the abandonment of cotton on some plantations.

Because of the failure of Tanguis on heavily infested soils the planters have been forced to turn to other crops, such as potatoes, beans, corn, and alfalfa, none of which is so profitable as cotton would be were it not for wilt. While this change of crops probably will reduce the amount of wilt in the soil, the chances of effectively controlling it by means of rotations are somewhat lessened by the fact that any crop grown must be irrigated, and under the present system of irrigation it is seldom possible to obtain water that has not passed over wilt-infested fields, thus constantly bringing in fresh inoculum.

2. Powdery mildew (*Erysiphe Malachrae* Seaver). This fungus, which has been described elsewhere (3), is prevalent on cotton in the coastal valleys. Although it may sometimes cause partial defoliation, this occurs late enough in the season so that little or no damage results.

3. Areolate mildew (*Ramularia arealo* Atk.) has been observed on *Gossypium peruvianum* in experimental plots at the Lima experiment station but not on Tanguis or the American varieties.

4. In 1929 the Alternaria leaf spot killed 5 acres of cotton seedlings near Lima.

CEREALS

1. Stripe rust of wheat (*Puccinia glumarum* (Schmidt) Eriks. & Henn.) occurs not uncommonly in the sierra where wheat is grown commercially, but it was not observed in the fields of the experiment station at Lima where *P. graminis* and *P. triticina* were always present.

Stripe rust is of much less importance in the country as a whole than stem rust. However, it has been observed causing leaf injury in some sections where both stem and leaf rust were absent. In June, 1930, fields of "Australian" wheat (variety not known), growing at an elevation of 13,000 on Cachi-Cachi farm near Jauja, were found in which stripe rust was prevalent and causing some leaf drying, while neither leaf nor stem rust was present.

2. The importance of stem rust in Peru has already been reported (2). While on trips to the sierra wheat-growing sections the writer has been interested in observing the gradual decrease in rust infection on ascending to elevations greater than 8,000 and 9,000 feet above sea-level. At Tarma (8,000 to 9,000 feet) in June, 1930, the percentage of infected plants of the Peruvian variety "Barba Blanca" was 100 per cent and the injury classed as medium. Ascending the mountain on the road from Tarma to the Huancayo Valley (10,000 to 11,000 feet) fields were examined along the way, in which the decrease in the degree of infection was obvious. In the Huancayo Valley the average percentage of infection was less than 30 per cent, the injury being classed as slight, while at Cachi-Cachi farm (13,000 to 14,000 feet) near Jauja not a trace of stem rust was found in fields extend-

ing over several hundred acres. This was found true also in 1929 during a trip to the same section.

A similar condition was observed in southern Peru in February, 1928, although, at that time, it did not appear so significant until found generally true in the wheat-growing sections of the country. Stem rust in the Arequipa Valley (8,000 to 9,000 feet) was extremely severe, the infection in every field examined being 100 per cent); whereas in the Cuzco Valley (10,000 to 11,000 feet) and at Juliaca (12,000 feet) only scattering plants of the same variety of wheat growing at Arequipa were rusted.

It must be remembered, of course, that wheat, growing at different elevations at a given time of the year, is in a different stage of maturity, a fact which might influence the degree of rust infection. February is the harvest month at Arequipa, while, at Cuzco, wheat usually begins to head during the early part of that month and is harvested in May and June. However, the small amount or absence of rust in the fields at harvest time at elevations above about 10,000 feet has been verified by later visits to the Huancayo-Jauja sections, and by reports from farmers in southern Peru, accompanied in some cases by specimens of the straw.

In a previous paper (2) the opinion was expressed that wind-blown spores from the mountainous wheat-growing sections were the source of stem-rust infection on the coast where wheat always suffers so severely from this disease that it rarely reaches maturity. There appear to be three possible sources of infection: (a) presence of an alternate host which bridges the gap from one crop season to the next; (b) survival of urediniospores on old straw; and (c) wind-blown spores from the higher altitudes.

Species of Berberis do not grow along the Peruvian Coast, although they are native to many sections of the sierra. However, infection of these plants by the stem-rust fungus has not been observed by the writer, even where they were growing adjacent to fields of heavily rusted wheat. No evidence has been accumulated to indicate that the species of Berberis native to Peru play a part in the life cycle of stem rust in that country.

The possibility of some other grass harboring the fungus was considered, but a collection of wild-grass rusts in the valleys adjacent to Lima failed to reveal the presence of *Puccinia graminis*. It seems improbable that a secondary host plant is responsible for initiating the epiphytotics of rust that occur when wheat is planted on the coast.

To determine whether the urediniospores survive from one crop to the next on the coast a series of inoculation and germination experiments was undertaken at the harvest season in November, 1928, and continued in 1929. Small sheaves of the varieties Pusa-4, Hope, and Khapli emmer, showing abundant uredinia, were gathered in the field on November 8, when the heads were in the hard-dough stage, and placed in screened cages in a

shaded place near the edge of the field. A few pieces of straw of each were taken to the laboratory to be used as a source of inoculum. Inoculations were made by dusting the urediniospores on seedlings of Pusa-4 showing the third leaf. Pusa-4 was selected because of its ready susceptibility to rust in the field. The small flower pots, in which the seedlings were growing, were then placed in a moist atmosphere under bell jars and incubated at 18 to 20° C. for 48 hours. At the end of that time the bell jars were removed and the pots, including uninoculated checks, were set in an open patio where they received only the morning sun. Readings for infection were made 15 days after inoculation. This inoculation procedure was repeated at the end of 15 and 30 days from the time of collection of the material in the field, with the straw from the cages serving as the source of inoculum.

The viability of the urediniospores from the three varieties at the time of collection was shown by the production of well-developed uredinia on the Pusa-4 seedlings in 15 days. From the material that had been stored 15 days in the cages, however, only weakly developed uredinia or yellowish spots were produced, while at the end of 30 days' storage there was no indication of infection. Similar results were obtained with Khapli emmer collected in the field on November 15 and December 1.

The experiment was repeated during November and December, 1929, and January, 1930. In no case did the urediniospores survive for more than 30 to 35 days, either when the straw was left in the field or placed in cages. Figures obtained with Khapli emmer are typical. The material was collected on January 13, 1930, from plants in the hard-dough stage and placed in cages as before. Field temperatures during the storage period varied from a daily minimum of 25° C. to a maximum of 36° C. The viability of the urediniospores was determined by germinating them in sterile distilled water in moist chambers at 20° C. and checking the results by inoculations of Pusa-4 seedlings at the time of collection and at the end of 10, 20, and 30 days. The germination counts in each case were based on the examination of 1,000 spores 3 to 3½ hours after placing them in the water. The results are shown in table 2.

From an initial germination count of 41.2 per cent the number of viable spores dropped to 20.5 per cent after 2 days and then decreased gradually

TABLE 2.—*Viability of urediniospores of Puccinia graminis tritici after different storage periods*

Days after collection	0	2	4	10	20	30	38
Percentage of germination	41.2	20.5	13.0	9.0	4.4	1.0	0
Infection of wheat seedlings	+			+	Weak		—

until only 1 per cent at the end of 30 days. After 38 days there was no germination.

Checking these results by plant inoculations it was found that numerous, well-developed uredinia were produced by the freshly collected inoculum. At the end of 10 days' storage of the inoculum the resultant uredinia were of normal appearance, although less numerous. After 20 days only scattered, small uredinia appeared, and at the end of 30 days there was no evidence of infection. Similar results were obtained with inoculum from Hope and Pusa-4.

From these results it seems improbable that the urediniospores remain viable from December or January until the following August when the first rust infection usually appears in the field. Attempts to germinate the spores from the old straw which had remained in the field during that time were not successful.

By the process of elimination wind-blown spores from the higher altitudes are left as the most probable source of stem-rust epiphytotics on the coast. While there is no experimental evidence to support this view, it does not seem unreasonable to believe that they cause at least the initial infections when it is considered that wheat is growing throughout much of the year at different elevations in the sierra and that rust spores are carried long distances by winds. In the case of wheat, growing in the Rimac Valley, it would mean a transfer of only about 60 miles. Considerable wheat is grown every year at Canta, 60 miles distant and 8,000 feet higher, the harvest season coming in June, when the crop is approaching the heading stage in the valley below.

3. It is interesting to note that *Puccinia graminis avenae* Eriks & Henn. was collected by the writer only once in Peru, a single heavily infected specimen from an experimental farm at Huaraz (elevation 11,000 feet) being collected in June, 1930. This was on an unnamed American variety.

At the Lima experiment station several American varieties of oats were grown for three seasons, and, although stem rust was severe on adjacent plots of both wheat and barley, it did not appear on the oats. Nor was the disease observed on oats in other sections of the sierra where stem rust was present on both wheat and barley. The fact that oats is a comparatively new crop in Peru and not yet generally cultivated in the cereal-growing regions may account for the limited distribution of the disease.

4. Barley stripe (*Helminthosporium gramineum* (Rab.) Eriks.) and spot blotch (*H. sativum* P., K., & B.) are common on barley in Peru, the former sometimes causing premature death of the plants and resulting in reduced yields. The severe injury attributed to *H. teres* Sacc. in a previous paper (1) was later found to be due to *H. gramineum*. Net blotch occurs in Peru, but it is of less importance than stripe.

Helminthosporium sativum presents a particularly serious problem in Peru because of the severity with which it attacks Khapli emmer, the only cereal of the wheat group that, up to the present time, has shown any possibility of commercial cultivation on the coast, stem rust making impossible the cultivation of any of the 110 varieties of durum and common wheats thus far tested. During the seasons of 1928, 1929, and 1930 some experimental plots were so severely blighted that they failed to mature. The fungus was isolated from lesions on roots, nodes, culms, leaves, glumes, and seeds.

5. *Helminthosporium oryzae* Br. de H. has been identified as the cause of "black point" of rice in several valleys of the coast.

6. *Phyllachora maydis* Maubl. is common on corn on the coast and sometimes is severe enough to cause premature drying of the leaves.

7. *Phoma zeicola* E. & F. is of rare occurrence on corn.

POTATOES

1. Late blight (*Phytophthora infestans* (Mont.) de By.) is of less "universal" occurrence in the country than was believed at the time the previous report (1) was made. It has since been determined by personal observation that essentially all of the damage attributed to this disease in the sierra is actually due to low temperatures. Local growers do not always recognize this fact and give descriptions of frosted plants which closely approximate the symptoms of blight. The term "hielo" is used for both blight and the effects of cold weather.

In a rather extensive survey of the potato districts of the country during 1928, 1929, and 1930 late blight was not found affecting either wild or cultivated species of Solanum at elevations above about 9,000 feet. In 1928, in the Arequipa Valley (8,000 to 9,000 feet), a small amount of blight was found in some fields. This appears to be contrary to what would be expected if blight were indigenous to Peru, a supposition which had generally been accepted as fact until the appearance of Reddick's paper (5). The disease is present and is often severe along the coast, particularly in the vicinity of Lima where introductions of seed potatoes from Europe and the United States have been made frequently in past years. The fact that blight apparently does not occur in the sections where the potato is native and where introductions of seed stock from other countries have been very infrequent, while the disease is common in the lowlands where such introductions have been made and where the potato is not native, lends some support to Reddick's theory that the blight fungus is not indigenous to South America. That the failure of the disease to develop at the higher altitudes is not due to the resistance of the varieties grown there is shown by their susceptibility to it when grown on the coast.

2. The widespread distribution of powdery scab (*Spongospora subterranea* Wallr.) in Peru has been reported previously (1). The writer has found the disease in every potato-growing section of the sierra visited, which includes the Departments of Arequipa, Cuzco, Puno, Ayacucho, Huancavelica, Junín, Huánuco, and Lima, while specimens were received at the laboratory from Apurimac, Ancash, Libertad and Cajamarca. Not a single field has been examined at harvest time in the Departments mentioned where the disease was not present, usually affecting at least 10 per cent of the crop and frequently 50 per cent or more. Fields on the larger farms, as well as on the small, isolated, mountain-side plots of the Indians, who probably have never grown anything but native potatoes, were affected. It seems to be expected by most growers that at least 5 to 10 per cent of the crop will be discarded each year because of the canker stage of the disease.

The fact that powdery scab is present throughout the parts of the country where the potato is native, particularly in the isolated section of the Andes where it is doubtful whether the Indians have ever had more than casual contact with the White Man, indicates that the disease is indigenous to the country. It is extremely unlikely that an introduced disease would have become so universally distributed throughout the Andean regions. Further support of the belief that it is indigenous was obtained on June 14, 1930, when a wild-potato plant with several tubers showing the characteristic skin lesions of the disease was found on Cachi-Cachi farm near Jauja. The plant was growing on a hillside which had never been cultivated so far as known and about three miles from fields of cultivated potatoes.

3. The presence of wart (*Synchytrium endobioticum* (Schilb.) Perc.) in Peru has been reported (1). From the time the disease was first discovered on Cachi-Cachi farm in September, 1928, a search was made in all potato-growing sections of the country visited in order to determine its distribution. In addition, frequent inspections were made of potatoes in the Lima public markets which had come from many different parts of the country.

No authentic case of the occurrence of wart outside of the vicinity of Cachi-Cachi farm has come to the writer's attention, although a grower from Puno (extreme southern Peru), on examining a specimen of the disease in the laboratory of the experiment station at Lima, stated that it occurred there.

At Cachi-Cachi wart appeared in 1928 and 1929 but was not found in the 1930 crop. The owner of the farm was unable to say how long the disease had been known in that section, but he was of the opinion that it had been there many years. The botanist of the agricultural school in Lima, who has been in Peru for 20 years, stated that Cachi-Cachi is the only place from which he has received specimens of wart.

4. Two rusts (*Aecidium cantensis* Arthur and *Puccinia Pittieriana* Henn.) occur on potatoes in Peru. The former is a new disease described by J. C. Arthur (4) from material collected by the writer in the vicinity of Canta, about 60 miles northeast of Lima, at elevations ranging from 8,000 to 9,000 feet. Although it is quite general on potatoes near Canta during the latter part of the growing season, it is of minor economic importance. It has not been observed outside of the Canta district. The disease also occurs on tomatoes.

Puccinia Pittieriana was collected in February, 1929, near Tarma, where it was causing some defoliation. It was not observed in other parts of the country.

5. Powdery mildew (*Oidium* sp.) occurs generally throughout the sierra, the heaviest infections being observed near Tarma and Canta. Sometimes the stems and petioles are covered by a whitish, powdery growth of the fungus, causing brown patches on the epidermis and often resulting in partial defoliation. The perfect stage of the fungus was not observed.

6. Stem rot and black scab (*Corticium vagus* Berk. & Curt.) are serious on potatoes on the coast nearly every season and frequently cause losses in the sierra. The yellow-flesh variety appears to be particularly susceptible. During the 1929 season some fields of this variety in the Rimac Valley were a total loss as the result of stem rot. No control measures are practiced.

The yellow-flesh variety is one of the choicest of Peruvian potatoes, and, because of its small yield, commands a price two to four times that of other varieties throughout the year. While it does not appear to be a heavy yielder, compared with other varieties, even when free of disease, diseases commonly cause considerable reduction in the yields obtained from it. The most important are stem rot, late blight, and mosaic.

7. Tuber rots, caused principally by species of Fusarium (*F. martii-minus* Sherb. and *F. solani* Ap. & Wr.), prevent the storage of potatoes for any length of time on the coast, particularly during the warmer months. For this reason the coast-grown crop always goes directly from the field to the public markets, and even during the short time intervening between harvesting and consumption considerable numbers of tubers are lost through decay. Cold-storage facilities are lacking. The sierra-grown crop usually is stored in sod houses where the temperature can be kept at a desirable point until market conditions are favorable on the coast.

FRUITS, VEGETABLES, AND MISCELLANEOUS

Diseases of fruits not previously reported include Diplodia rot of oranges (*Diplodia natalensis* Ev.), which has been found infrequently on market fruit in Lima; blue mold of citrus fruits (*Penicillium italicum* Wehmer), which causes considerable loss of fruits in storage and shipment;

peach-leaf curl (*Exoascus deformans* (Berk.) Fckl.), which is serious in some of the sierra valleys, particularly Tarma and Ayacucho, where it is gradually killing small orchards; peach mildew (*Oidium leucoconium* Desm.), general on peaches, but of no economic importance; and a wilt of papaya, with which a species of Fusarium was found constantly associated.

Mosaic is frequently a serious disease of tomatoes. Very little of it was noted in 1928, but, during the 1929 season, losses estimated at 50 to 75 per cent of the crop resulted from it in the truck areas adjacent to Lima. In 1930 losses approached 50 per cent. Mosaic of peppers also is common and often assumes importance.

Miscellaneous diseases identified include *Cercospora apii* Fresen. on celery; *C. citrullina* Cke. on watermelon; *C. zebrina* Pass. on alfalfa; stem rot (*Corticium vagum* Berk. & Curt.) of radish, beet, celery, spinach, strawberry, buckwheat, soy bean, and broadbean; and the Oidium stage of powdery mildews on tomato, eggplant, pepper, cabbage, cauliflower, turnip, radish, cucumber, lima bean, soy bean, velvet bean, broadbean, cowpea, cassava, alfalfa, buckwheat, crimson clover, sour clover, sunflower, sweet pea, and zinnia.

SUMMARY

This paper lists 53 diseases of economic plants not hitherto reported from Peru, together with a discussion of several of the most important ones previously reported.

Sugar-cane mosaic has been found generally distributed on the plantations of the Carabayllo Valley, but it has not been observed in other sections of the country.

Cotton wilt is causing the abandonment of cotton culture in some sections. Field tests are reported in which the Peruvian variety Tanguis proved more resistant to wilt than several North American varieties.

Stem-rust infection of wheat in the highlands was found to decrease gradually with the elevation. Germination and infection experiments are reported indicating that the urediniospores do not survive from one crop to the next, on the coast, supporting the theory that rust epiphytotics on the coast are initiated by wind-blown spores from the sierra. Stripe rust of wheat sometimes causes injury at high elevations where stem and leaf rust are absent.

Potato late blight has not been found in the regions where the potato is native. Its absence from these sections and presence only where foreign seed stock has been introduced support Reddick's theory that it is not indigenous to South America.

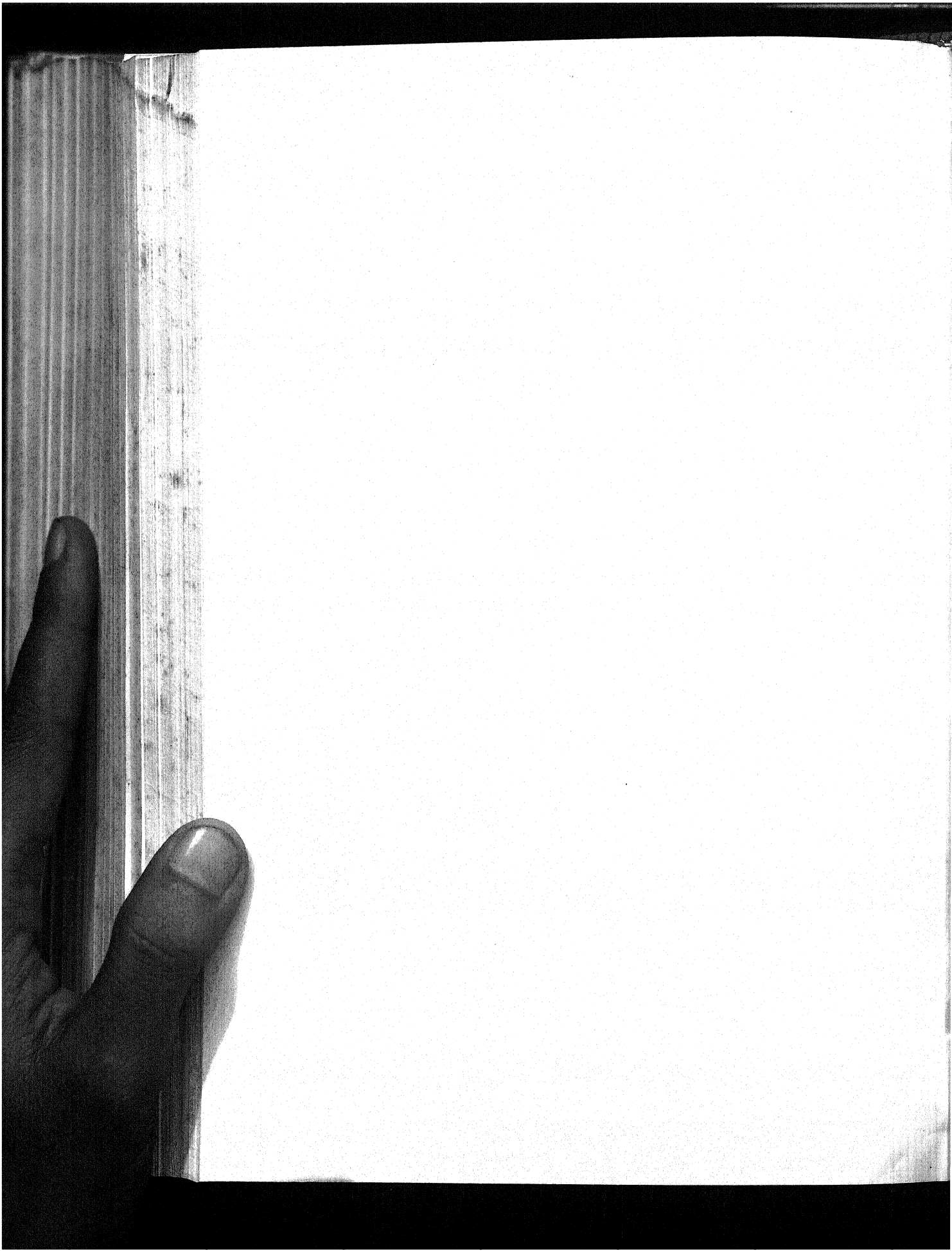
Potato powdery scab occurs throughout the Andes where the potato is native and was found once on wild potatoes, indicating that it is indigenous

to the country. Wart was observed in only one potato-growing district. Minor diseases of potatoes reported include a new rust.

HOUMA, LOUISIANA.

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DISTRIBUTION OF CERTAIN FUNGI IN COLORADO SOILS

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The fungus flora of our soils doubtless plays an important part in soil fertility, but in the arid regions of the West little study has been given soil organisms. The soils of Colorado differ greatly in moisture and soluble-salt content and offer a wide range of conditions for growth of fungi.

It is the purpose of this paper to continue the previous report of LeClerg and Smith (3) and give a further survey of the fungus flora from a number of localities and environments in an effort to ascertain the relative frequency of fungus species in this region in soils producing different crops.[2]

MATERIALS AND METHODS

Thirteen different soil types were examined, from which 226 samples were taken.

The methods employed in collecting, preparation of samples, and dilution were the same as described in a previous paper (3). Briefly these are as follows: All samples were taken either with a sterile trowel or a soil auger fitted with a steel sleeve. The sampler was sterilized with mercuric chloride (1:1,000). The top layer of soil, about 1 in., was removed with a sterile spatula before the sample was taken. The samples were carried into the laboratory in sterile, air-tight tin containers. Ten grams of each sample were placed in 300 cc. of sterile tap water and shaken thoroughly. Dilutions were made up to 1:30,000. One cc. of this dilution was poured into a sterile Petri dish, and the plates were poured in the usual way. Waksman's medium was used for all isolation work.

The writer is aware that all the fungi present in the soils examined were not obtained, since special methods are necessary to recover several forms, as Rhizoctonia and Pythium. The plate method for quantitative determinations of soil fungi has its limitations, as it does not distinguish between spores and mycelia. It shows, within limitations, the potential fungus content of the soils examined. The percentages given can not, therefore, be considered as being exact representations of the distributions of the different fungi but are indicative of the relative numbers of each in the different soils under the conditions of sampling, dilution, etc.

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[2] The writer wishes to express his appreciation to Dr. L. W. Durrell for furnishing facilities for this study and for reading the manuscript, and to Dr. J. C. Gilman of the Iowa State College for assistance in identification of the fungi.

DISTRIBUTION AND ABUNDANCE OF FUNGI IN DIFFERENT SOILS

During the course of this work 31 species of fungi were isolated. In confirmation of the previous work (3), species of Aspergillus and Penicillium were more prevalent than any other fungi isolated.

The data in table 1 show that in proportion to the number of isolations the greatest number of fungi found in any of the soil conditions examined was under red-clover plants.

TABLE 1.—*Number of fungi in each of 13 soils*

Kind of soil	Number of samples examined	Number of isolations	Number of species isolated	Total number of fungi isolated (Colonies)
Red clover	14	42	9	1350
Sugar beets	26	78	14	1119
Potato	30	90	10	1114
Wheat	15	45	19	629
Plowed	34	102	16	615
Corn	30	90	21	565
Cherry orchard	15	45	10	437
Gladiolus	10	30	6	431
Below surface (6–12 in.)	8	24	10	244
Alkali	19	57	7	218
Wind-blown	10	30	10	196
Below surface				
(3 ft.)	10	30	4	59
(6 ft.)	5	15	2	24

It is apparent from the data in table 1 that the nature of the cover crop or soil conditions results in modification of the population of soil fungi. The soils examined under such crops as red clover, sugar beets, gladioli, potatoes, and wheat seem to be associated with or make conditions favorable for a relatively large population of fungi. Under such conditions as corn and under cherry trees a smaller population of fungi was found.

The second highest fungal content was in soils producing sugar beets. This agrees quite closely with the results of Starkey (6), who, examining soils supporting apple trees, rye, corn, sugar beets, alfalfa, and eggplant, found the number of fungi to be greatest under alfalfa and second under sugar beets. Jasevoli (2), in Italy, found that soils devoted to potatoes had a higher fungus content than those producing fruit or grain. Similar results also were found in the work herein reported, under conditions in Colorado.

The data in tables 2 and 3, as a whole, suggest that certain crops and soil conditions may affect certain groups of fungi. Corn land contained 21 dif-

TABLE 2.—*Percentage of samples containing various fungi and relation of each fungus to total flora*

Name of fungus \ Soil type number[a]	Percentage of samples containing fungus													Percentage of total flora represented by each fungus										
	1	2	3	4	5	6	7	8	9	10	11	12	13	1	2	3	4	5	6	7	8	9	10	11	12
Absidia spinosa	7									70	80	20	29	b									53	71	3
Acrothecium robustum													3												
Alternaria spp.	17			30	7	79	20	5		10		60	b	2			2	b	5	4	b		5		8
Aspergillus clavatus	7		34			7	20					70	3	1		6			b	3					7
" *flavus*	3						7							b						b					
" *fumigatus*									13			20	3									b			4
" *glaucus*		3							13						2							3			
" *minutus*			11				13									3				8					
" *niger*	23	10	4		27			32						4	b	b		2			2				
" *terreus*	3	7	8											b	b	b									
" *wentii*				1					13								b								12
Cunninghamella verticillata		3	11				7						3		b	b				1					
Fusarium spp.	80	100	81	100	100	65	100	63	50	50	60	100	68	44	25	24	15	30	2	23	24	7	39	29	54
Gliobotrys alboviridis					1													b							
Hormodendrum cladosporioides	17	20	25	10	27	100	20					10	9	1	2	2	b	3	75	1					b
Mucor glomerula	7					21	6							1					2	b					
" *lausannensis*	3								13				3	b								b			
Penicillium chrysogenum	13					64	27							20					1	20					
" *citrinum*	10		4	60	13		13						26	2		b	38	5		b					
" *digitatum*	13		42		20		27					40	6	2		48		2		14					12
" *echinatum*	7						13		13	10			18	b						3		1	5		
" *expansum*	20	100	35		87		13	58	88				41	4	54	14		42		14	44	73			
" *humicola*	10					14	13							1					b	3					
" *purpurogenum*							7													b					
" *stoloniferum*			8													b									
" *viridicatum*	10													2											
Phoma spp.	3						7					10	3	b						b					1
Rhizopus elegans	17	60			27		13	11						1	15			1		b	b				
" *nigricans*	50	33	4			28	40	5	25			30	47	6	1	b			b	1	1	2			2
Sepedonium chrysospermum			11													1									
Trichoderma lignorum	37	33	19	100	13	36	26	16	13			70	26	5	1	2	40	8	b	1	1	b			11

[a] Soil type number 1—Soil supporting corn.
" " " 2— " " potatoes.
" " " 3— " " sugar beets.
" " " 4— " " gladioli.
" " " 5— " " cherry trees.
" " " 6— " " red clover.
" " " 7— " " wheat.

[a] Soil type number 8—Alkali soil.
" " " 9—Below surface (6–12 in.).
" " " 10—Below surface (3 ft.).
" " " 11—Below surface (6 ft.).
" " " 12—Wind-blown soil.
" " " 13—Plowed soil.

[b] Less than 1 per cent.

TABLE 3.—*Distribution, occurrence, and abundance of fungi isolated from 13 different types of Colorado soils*

Name of fungus	Distribution		Occurrence		Abundance	
	Number of soils containing fungus	Percentage of soils containing fungus	Number of samples in which fungus occurred	Percentage of samples in which fungus occurred	Total number of colonies from 13 soils	Percentage of total flora represented by each fungus
Absidia spinosa	5	38	25	11	86	1
Acrothecium robustum	1	8	1	[a]	1	[a]
Alternaria spp.	9	69	32	14	115	2
Aspergillus clavatus	6	46	24	10	46	1
" *flavus*	2	15	2	[a]	2	[a]
" *fumigatus*	3	23	4	2	11	[a]
" *glaucus*	2	15	2	[a]	27	[a]
" *minutus*	2	15	5	2	88	1
" *niger*	5	38	20	9	100	1
" *terreus*	3	23	5	2	5	[a]
" *wentii*	2	15	2	[a]	30	[a]
Cunninghamella verticillata	4	31	6	3	20	[a]
Fusarium spp.	13	100	181	80	1596	23
Gliobotrys alboviridis	1	8	2	[a]	2	[a]
Hormodendrum cladosporioides	9	69	44	19	1105	16
Mucor glomerula	3	23	6	3	28	[a]
" *lausannensis*	3	23	3	1	3	[a]
Penicillium chrysogenum	3	23	17	8	443	6
" *citrinum*	6	46	22	10	317	5
" *digitatum*	6	46	28	12	641	9
" *echinatum*	5	38	12	5	69	1
" *expansum*	8	62	92	40	1422	20
" *humicola*	3	23	7	3	29	[a]
" *purpurogenum*	1	8	1	[a]	1	[a]
" *stoloniferum*	1	8	2	[a]	11	[a]
" *viridicatum*	1	8	3	1	12	[a]
Phoma spp.	4	31	5	2	11	[a]
Rhizopus elegans	5	38	31	14	186	2
" *nigricans*	9	69	58	26	106	1
Sepedonium chrysosperum	1	8	3	1	14	[a]
Trichoderma lignorum	11	85	67	30	353	5

[a] Less than 1 per cent.

ferent forms comprising 4 different Aspergilli and 7 different Penicillia. It was found that gladiolus soils contained the lowest number of different species of fungi, if consideration be confined to soils supporting only economic crops. In the samples from these gladiolus soils 6 different species of fungi were obtained, including 1 species each of Aspergillus and Penicillium. Species of Fusarium were found to be the most prevalent fungi in all samples taken from wind-blown soils and from soils supporting potatoes, gladioli, cherry trees, and wheat. They were present also in a majority of the samples taken from the other soil types.

From the data in table 3 it is evident that species of Fusarium were widely distributed, occurred more frequently than any other fungus, and constituted 23 per cent of the total flora found in all of the samples. *Penicillium humicola* Oudem. ranked second and *Hormodendrum cladosporioides* (Fr.) Sacc. third. The 8 species of Aspergillus and the 9 species of Penicillium outnumbered any of the other fungi isolated.

On the succeeding pages a discussion is given of the distribution and abundance of fungi in the 13 different soil types examined.

Sugar-beet-producing soils.—The samples of sugar-beet soils were collected from different parts of northern Colorado. Some were taken near Loveland where the soil was white with alkali and very heavy and black; others, east of Greeley; and some, north of Fort Collins.

From the 26 samples examined 14 different species of fungi were isolated. *Sepedonium chrysospermum* (Bull.) Fries was found only in sugar-beet soils. It was present in 11 per cent of the samples but was not abundant, constituting less than 1 per cent of the entire flora in these soils. This fungus has previously been found only twice in the soil, once in England (1) and once in New Jersey (8).

Species of Aspergillus and Penicillium were widely distributed in beet soils. *Penicillium digitatum* Sacc. and *P. expansum* (Link) Thom were most abundant. Fusarium was the most widely distributed fungus in these soils, it having been found in 81 per cent of the samples collected. It constituted about 24 per cent of the entire fungus flora.

Gladiolus-garden soils.—The samples examined from gladiolus soils were taken from fields that had produced plants showing marked signs of root rot (*Fusarium* sp.) the previous year. These fields were located about 10 miles west of Denver, Colorado, in a district where gladioli are extensively grown.

Six different species of fungi were isolated from these soils. Species of Fusarium were found in all the samples examined and constituted 30 per cent of the entire fungus flora. Likewise, *Trichoderma lignorum* (Tode) Harz. was distributed in each sample and formed 40 per cent of the total number of fungi isolated. Species of Alternaria were not abundant (2 per

cent of the total flora) but were found in 30 per cent of the samples. Only one member of the genus Penicillium (*P. citrinum* Thom) was isolated, but it was generally distributed (60 per cent of the samples) and formed 38 per cent of the entire flora obtained.

Corn-producing soils.—Samples were collected in Colorado near Fort Collins, Loveland, Timnath, and Greeley during January and February of both 1927 and 1928. In one field the subsoil was frozen, but the surface layer had thawed during a warm period. None of the samples was taken from frozen soils.

These soils contained more different species of fungi than any soils examined. Twenty-one species were isolated. The total number of fungi found in these soils was small, averaging 6 colonies per isolation.

Species of Fusarium were the most abundant and widely distributed fungi in soils supporting corn. These fungi were found in 80 per cent of the samples and constituted 44 per cent of the total flora. There were 7 species of Penicillium and 4 species of Aspergillus represented.

Potato-producing soils.—The samples of potato soils were all collected on March 13, 1928, at Greeley, Colorado, in two different fields. Part of them (20 samples) were taken from a field that previously had produced a crop of potatoes in which a high percentage of the plants had been attacked by Fusarium wilt and 10 samples were obtained from a field that had produced relatively disease-free potato plants.

Ten different species of fungi were isolated from the 30 samples. *Fusarium* spp. and *Penicillium expansum* were found in every sample examined. The former constituted 25 per cent and the latter 54 per cent of the total flora. The other fungi found were *Aspergillus glaucus* Link, *A. niger* van Tiegh., *A. terreus* Thom, *Cunninghamella verticillata* Paine, *Rhizopus elegans* Eidam, *R. nigricans* Ehrenb., *Trichoderma lignorum,* and *Hormodendrum cladosporioides.* This group of fungi constituted less than 25 per cent of the entire flora.

More colonies of *Fusarium* spp., per culture, were obtained from the soil that had produced a "wilt-sick" crop than from the soil that had produced a crop of only a few affected plants.

Cherry-orchard soils.—Fifteen samples were collected near Loveland, Colorado, on February 8, 1928, from two different cherry orchards. Ten species of fungi isolated from these soils consisted principally of *Fusarium* spp. and *Penicillium expansum.* The former were present in all the samples examined and the latter in 87 per cent of the samples. *Fusarium* spp. formed 30 per cent of the total flora, while *P. expansum* constituted 42 per cent of the total number of fungi found. The remaining fungi isolated consisted of *Alternaria* spp., *Aspergillus niger, Gliobotrys alboviridis* v. Höhnel, *Penicillium citrinum, P. digitatum, Rhizopus elegans, Trichoderma lignorum,* and *Hormodendrum cladosporioides.*

Wheat-producing soils.—Fifteen soil samples were collected from widely distributed places in Colorado in fields of winter wheat. Collections were made at Fort Collins, Rocky Ford, Akron, and Greeley.

Nineteen species of fungi were isolated from the 15 samples of wheat soils. Of this number about one-third (7) consisted of species of Penicillium. Species of Fusarium were found in all the samples and constituted 23 per cent of the total flora. Species of Alternaria were found in one-fifth of the samples but formed only 4 per cent of the total number of fungi. Species of Penicillium were widely distributed, and the following were isolated: *P. chrysogenum* Thom, *P. citrinum, P. digitatum, P. echinatum* Dale, *P. expansum, P. humicola* Oudem., and *P. purpurogenum* Stoll. About 33 per cent of the soil flora consisted of *Aspergillus clavatus* Desm., *A. flavus* Link, *A. minutus* Abb., *Cunninghamella verticillata, Mucor glomerula* (Bain.) Lendn., *Phoma* sp., *Rhizopus elegans, R. nigricans, Trichoderma lignorum,* and *Hormodendron cladosporioides.*

Alkali soils.—Some of the alkali soils from which samples were taken had only a vegetative cover of *Sporobolus airoides* Torr., which is indicative of a considerable amount of alkali in the soil. Another field supported a dense cover of *Distichlis stricta* (Torr.) Rydb., which indicates very high alkali content. Nineteen samples were collected from different fields all of which were high in soluble salts.

The 57 isolations from these soils developed only 218 colonies of fungi, or an average of 4 colonies per isolation. This would seem to indicate that the presence of salts in the soil tends to interfere with the growth activities of the soil fungi. Seven different species were isolated from the 19 samples. It is interesting to note that *Fusarium* spp. were very abundant; 63 per cent of the samples contained species of this fungus and constituted 24 per cent of the total flora isolated from alkali soils. *Penicillium expansum* also was abundant, appearing in 58 per cent of the samples and forming 44 per cent of the total flora. Other fungi isolated from alkali soils were: *Alternaria* spp., *Aspergillus niger, Rhizopus elegans, R. nigricans,* and *Trichoderma lignorum.*

Subsurface soils (6–12 in. deep; 3 ft. deep; 6 ft. deep).—The average number of fungi per isolation decreased with depth; at 6–12 in., 10 colonies were found; at 3 ft., 2 colonies; and at 6 ft., only 1 colony per isolation appeared on the agar medium. Other workers, Russell (5), Paine (4), Taylor (7), and LeClerg and Smith (3), have found this to be the condition in soils below the surface.

The samples taken at 6–12 in. showed that *Penicillium expansum* and *Fusarium* spp. were the most abundant. Other fungi found were: *Aspergillus fumigatus* Fr., *A. glaucus, A. wentii* Wehm., *Mucor lausannensis,* Lendn., *Penicillium echinatum* Dale, *Rhizopus nigricans,* and *Trichoderma lignorum.*

Four different species were isolated 3 ft. below the surface. These were *Absidia spinosa, Alternaria* spp., *Fusarium* spp., and *Penicillium expansum*. Species of Fusarium and *Absidia spinosa* were found most frequently and abundantly at this depth.

Absidia spinosa and *Fusarium* spp. were isolated at a depth of 6 ft. The former was most abundant.

Wind-blown Soils.—Ten samples of wind-blown soils were collected near Akron and Sterling, Colorado, on May 1, 1928. The soil contained very little organic matter and did not have a vegetative cover.

Species of Fusarium were found in all samples of these soils and constituted over half (54 per cent) of the total fungi isolated. *Trichoderma lignorum, Aspergillus clavatus,* and *Alternaria* spp. were very abundant. Besides these fungi 6 other species were obtained, which included *Absidia spinosa, Aspergillus fumigatus, Penicillium digitatum, Phoma* spp., *Rhizopus nigricans, Trichoderma lignorum,* and *Hormodendrum cladosporioides.*

Plowed Soils.—Sixteen different species were isolated from various plowed soils. The most prevalent species found were members of the genus Fusarium. *Penicillium expansum* and *Absidia spinosa* were also abundant. The other fungi isolated consisted of *Acrothecium robustum* Gilm. and Abb., *Alternaria* spp., *Aspergillus clavatus, A. fumigatus, Cunninghamella verticillata, Mucor lausannensis, Penicillium citrinum, P. digitatum, P. echinatum, Phoma* spp., *Rhizopus nigricans, Trichoderma lignorum,* and *Hormodendrum cladosporioides.*

SUMMARY

A study was made of the fungus flora of 13 soils of different physical conditions supporting different crops. Thirty-one species of fungi were isolated. Alkali salts tend to reduce fungus flora, although *Fusarium* spp. were abundant in this type of soil.

Fungi were more abundant in planted than in nonplanted soils, such as wind-blown soils.

The number of fungi was greatest in soils producing red clover.

The only forms that occurred under all soil conditions were *Fusarium* spp. These fungi were more abundant in soils that had produced wilt-sick crops of potatoes than in soils that had produced a crop of only a few affected plants. Likewise, they were very abundant in soils that had produced a crop of gladioli badly affected with Fusarium root rot.

Species of Aspergillus and Penicillium outnumbered any other fungi isolated.

The number of fungi decreased with depth into the soil.

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NODULE PRODUCTION ON ETIOLATED VETCH SEEDLINGS

J. K. WILSON

Descriptions of the mode of entry of the legume bacteria into the root tissue of leguminous plants have appeared from time to time in the literature. They agree for the most part in details. Apparently, the bacteria produce a small colony near the apical end of a root hair. Here they readily effect entrance through the cell wall and mingle with the protoplasm. Soon they develop an infection thread. This extends towards the main root. By this time, usually within one day, the root hair had enlarged and on certain plants become twisted. Further progress of the infection thread into other cells, together with increased cell division, produces a nodule.

In 1902 Peirce[1] published an article on the root tubercles of bur clover and of some other leguminous plants. In this he described the infection of root hairs on seedlings whose roots were only an inch or so long. The description agreed with that that had been given for the infection of root hairs of other plants. From the experiments performed it was evident that infection occurred before the seedlings were old enough to carry on photosynthesis. This indicated that infection may occur without the stimulus of photosynthesis and that the latter may not be necessary for nodules to develop.

Perhaps the reserve material in the seed sufficed to carry the seedling through the infection period and, if enough assimilable carbon compounds were available, either as reserve materials in the seed or supplied to the seedling, macroscopic nodules would develop in the absence of light. Definite experiments, therefore, were started, the object being to produce nodules on etiolated seedlings.

A medium in which to grow the plantlets contained the following:

$MgSO_4$	0.2 gram
KH_2PO_4	0.2 "
NaCl	1.0 "
$CaSO_4$	0.1 "
Powdered agar agar	15.0 grams
$CaCO_3$	excess
Water	1 litre.

After the above medium was boiled for a few minutes 50-cc. portions were run into test tubes 4 x 16 cm. in size. These were then divided into

[1] Peirce, G. J. The root-tubercles of bur clover (*Medicago denticulata* Willd.) and of some other leguminous plants. Calif. Acad. Sci. Proc., Ser. 3 (Botany) 2: 295–328. 1902.

lots. Each lot received a certain sugar in definite concentrations. The sugars used were dextrose, levulose, and saccharose, and the concentrations were 0.50, 1, and 2 per cent of each, respectively. Twelve tubes were provided without sugar. Each was plugged and all were sterilized.

After sterilization, but before the contents of the tubes had cooled, enough sterile calcium chloride solution was added to make a concentration of this salt in the medium 1/500 molecular. The contents were thoroughly mixed and the tubes placed in an upright position until they were cold.

On October 6 seed of vetch, *Vicia villosa* Roth., were sterilized with a solution of calcium hypochlorite and 5 or 6 of them dropped on the surface of the agar medium in each tube. Two days later some growth from an active culture of *Rhizobium leguminosarum* Frank was suspended in sterile water and a few drops added to each tube. These tubes were then placed in an incubator at 25° C.

No daylight was allowed on the seedlings during the growth period but they were examined periodically under the electric light. Plants grown without access to sugar died much sooner than plants that had access to 0.50 per cent saccharose, while those that grew where 2 per cent saccharose was supplied not only lived the longest but also produced the largest plants. In several instances the plants grew to 30 or 40 cm. in length. All plants were entirely etiolated. Root development also was very good.

It was evident from an occasional examination of the plant roots, which could be seen through the test-tube wall, that infection had occurred and nodules were developing on a few plants. After 36 days, when it was evident that many plants were drying, the test tubes were removed from the incubator and the plants taken from them. The roots were carefully examined. Plants grown without access to sugars showed very few nodules. Out of 40 or more plants only 1 nodule was found. Also where levulose 0.50 per cent concentration, was supplied to the plants only a single nodule was found. Plants with access to dextrose also failed to develop nodules in all the concentrations except that of 2 per cent. Here 1 plant had 2 nodules. Plants grown with saccharose in the medium developed nodules in all the concentrations supplied, more being present in the 0.50 and 1 per cent concentrations than in the 2 per cent concentration. Some plants that were grown in the 0.50 per cent sugar medium had as many as 4 nodules. Figure 1 shows the appearance of the macroscopic nodules of etiolated vetch plants in a tube.

This experiment has been repeated several times with the same results. It has also been tried with peas, alfalfa, and red clover. No nodules were seen on the two latter plants but 2 nodules were found on a pea plant that had grown with access to 1 per cent dextrose. Many hand sections of the nodules from these plants were examined under the microscope. The nodular cells were packed with bacteria.

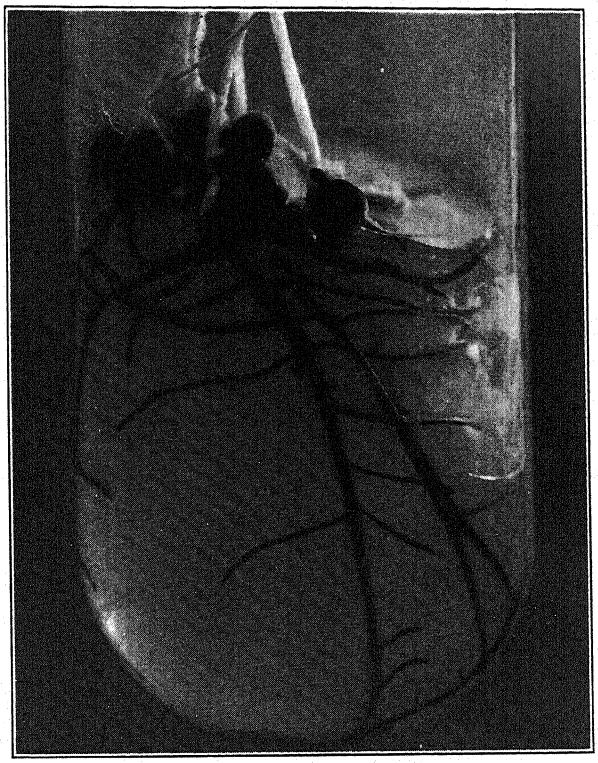


FIG. 1. Etiolated vetch seedlings showing nodules on roots. Grown for 36 days with 1 per cent saccharose in medium.

The foregoing observations, together with those recorded by Peirce, suggest that infection of leguminous plants by Rhizobium may occur before photosynthesis begins and that, if suitable conditions are maintained by supplying a satisfactory carbohydrate to the seedling, macroscopic nodules may develop in the absence of light.

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PHYTOPATHOLOGICAL NOTES

The Viruses Concerned in a Natural Epiphytotic of Streak in Tomatoes.—While viruses and virus combinations are known which will cause streak in tomatoes experimentally, the identity of the viruses concerned in natural streak has been determined and reported in but few cases. This note is for the purpose of recording the viruses concerned in a natural epiphytotic of streak and mosaic which occurred in the horticultural greenhouse at the Kentucky Agricultural Experiment Station during the fall of 1930. Tomatoes have been grown in this house during the past several years, but no cases of mosaic developed until the past fall. One year a few tomato plants were found affected with the so-called healthy-potato virus, the source seeming to be Cobbler potatoes, growing in the house.

Streak was first noticed about the middle of October, 1930, when the plants were approximately 3 ft. high. It started near one corner of a bench and appeared to spread gradually so that about 4 weeks later, of 51 plants in this bench, 22 were affected with streak and 12 with mosaic. Several plants in the adjoining bench were affected with streak and mosaic. In house 2, separated only by a partition in which is a door, no streak developed, but mosaic was present in many plants.

Inoculations were made to 59 Turkish tobacco plants from each of the 51 plants in the first bench of house 1 and from selected plants in bench 2 and in house 2. The tobacco plants, following infection, could be placed in 4 groups as follows:

Healthy	15
Healthy-potato	3
Tobacco-mosaic	18
Tobacco-mosaic with necrosis	23

The healthy-potato virus was obtained from plants with a very faint but distinct mottle, quite typical of the disease produced when tomatoes are inoculated with this virus from potatoes. The disease in tobacco was characterized by small, concentric rings on rubbed leaves and necrotic ring and line patterns and watered-silk chlorotic patterns in the newer leaves. It was found only in house 1. The tobacco mosaic was obtained from plants having a rather severe mosaic, but no evidence of streak on the stalk or petioles and no leaf necrosis. When dried, the virus retained its infectivity, indicating that it is one of the typical tobacco mosaics. It produced a severe mosaic in tobacco, without leaf necrosis, on rubbed (inoculated) and subsequent leaves. The necrotic mosaic was obtained from some slow-growing plants that appeared to have only tobacco mosaic or mosaic with some leaf necrosis and from rapidly growing plants with

stalk and petiole streak and leaf necrosis. Fruits on these plants developed no mottling nor necrosis. In tobacco, necrotic spots developed on rubbed leaves in a few days, the spots gradually increasing until many of the inoculated leaves died. Gradually the symptoms of typical, severe tobacco mosaic developed in the growing point, accompanied by necrotic dots or small rings. These plants usually were more stunted than those with mosaic only. Mosaic, when transferred to tomatoes, produced mosaic only. The viruses obtained from mosaic and healthy-potato tobacco plants, respectively, produced streak in tomatoes identical with that produced by transfers from the necrotic mosaic-tobacco plants to tomatoes. It was shown in the following ways that streak in the tomatoes and the necrotic mosaic in tobacco were the result of a mixture of tobacco mosaic and the potato virus. 1. A mixture of the two viruses produced symptoms in tobacco and tomatoes identical with those produced by the virus complex from streak tomatoes. 2. Symptoms of the healthy-potato virus could be clearly recognized in tobacco plants affected with the streak virus complex. 3. Transfers of the streak virus complex to virus-free seedling potatoes (previously tested for the healthy-potato virus and found free) produced stem and leaf streak typical of that produced by tobacco mosaic alone, but transfers to tobacco from potato leaves produced subsequent to inoculation resulted in only the concentric rings and other symptoms typical of the healthy-potato virus. 4. Transfers of the streak virus complex to *Datura stramonium* resulted in a mild mottle of the new leaves and transfers from these to tobacco resulted in the healthy-potato disease. Thus the healthy-potato virus was separated from the virus mixture and obtained pure in tobacco. The evidence seems to indicate that these two viruses only were concerned in this natural epiphytotic.[1]

As to the source of the viruses, nothing is known definitely. It is probable that they were introduced into the house independently, as the potato virus was present alone in a number of tomato plants. It is not likely that this would have happened if the viruses had come into the first plant together, as tobacco mosaic is so readily spread that mechanical operations which would spread the potato virus would almost certainly spread the tobacco-mosaic virus. The fact that streak was found in one house and only tobacco mosaic in the adjoining house suggests that mosaic was spread from the first house to the second on the hands of the caretakers, probably while suckering, and that the potato virus was not spread in this way. Tomato plants out of doors were affected with various kinds of mosaic, and,

[1] Following removal of the tomato plants in midwinter, the benches were not reset with tomato plants until about a month later when virus-free seedlings were set. A number of these became infected with tobacco mosaic, evidently from nondecomposed roots of the previous crop, but streak and the healthy-potato disease did not reappear.

as the men who handled these also handled the greenhouse tomatoes, the mosaic virus could have been introduced in this way.

The source of the potato virus and its spread in house 1 are not so readily explained. Potatoes were growing within a hundred yards of the house, and, while we have no evidence of insect spread of this potato virus, yet it is possible that it may have been introduced by insects to house 1 and spread either by man or by insects from plant to plant in this house before the introduction of the tobacco mosaic. Its early introduction seems probable, as the mottle symptoms were found well down on some of the plants, far below those of the streak or mosaic symptoms. Another possible means of introduction was on the hands of the men. The early-crop potatoes, dug at the usual time, rotted in the cellar and had frequently to be picked over during the summer and fall. The men who did this also cared for the tomatoes and may have introduced the potato virus in this way.—W. D. VALLEAU AND E. M. JOHNSON, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

A Rubber Dressing for Tree Wounds.—The experimenter who works with dressings for tree wounds finds himself confronted at the outset of his investigation with the dual nature of the task peculiar to wound dressing. By the application of various paints he hopes to accomplish two distinct purposes: (1) He endeavors to preserve the heartwood against the attack of weather, insect, and fungus. (2) He seeks to protect the region loosely designated as the "cambium" against drying out during at least the initial stages of callus formation, in order to assure prompt closing in of the ring of growing wood and bark that is ultimately to form about the wound. He has thus to deal with both living and dead tissues. Applications to living parts must be restricted to the use of more or less inert materials which do not penetrate the tissues deeply and which are nontoxic to the living cells. In dressing the inner area of the wound it is desirable to attempt the impregnation of the heartwood to some depth with preservatives which will be toxic to biological foes and deterrents to checking. Here, the heartwood being already dead, there is no question of toxicity to the cells.

In general practice the ringing of the cambium region with one material and the painting over of the wound area within with another, while long recognized as theoretically sound, are seldom attempted. It is more troublesome to apply two kinds of dressings than a single dressing. The entire cut generally is painted with a material, primarily designed (1) to preserve the dead tissues even though it injure the growing layer; or (2) to aid the growth of the callus and afford little protection to the heartwood; or (3) to seek a middle ground of giving reasonable protection to the dead tissues with a minimum of accompanying injury to the live.

During the past 4 years the writer tried the effect of a number of substances on the formation of callus with a view to testing their merits for preventing the drying out of the cambium and for protecting it when strong chemicals are applied to the heartwood. But few of the materials tested proved valuable. Shellac is applicable to this purpose, being a non-injurious material. It has the disadvantage of short life when not covered with some more permanent material, sometimes becoming useless through weathering after a few days of exposure. Its protective coating is thin and brittle. Melted beeswax is another beneficial dressing for this purpose. It coats the surface with a reasonably thick, firm layer that neither penetrates deeply nor kills the living cells. Results with it are superior to those obtained with shellac. Like the latter material, it becomes somewhat brittle with age. The fact that it must be kept hot while being applied is a further disadvantage.

From a strictly theoretical standpoint, rubber was thought to offer great promise for such ringing of wounds. One would expect to obtain by its use an elastic coating that would protect the growing layer from toxic preservatives painted onto the heartwood. Some years ago the writer attempted two methods of applying rubber to tree wounds: (1) By the cementing on with shellac of rubber dam similar to that used in dentistry and (2) by painting the surface with liquid-rubber compounds designed for the application of cold patches to inner tubes of automobile tires. Both methods were pronounced failures. The dam became loose, and the benzene used as a solvent in the liquid-rubber compound penetrated and injured the living cells.

Rubber latex,[1] containing vulcanized rubber suspended in a colloidal state, is now available on the market. It is a white liquid having much the color and consistency of thin cream. When the liquid is applied to a surface by means of an ordinary paint brush or a paint sprayer and allowed to dry, a film of cured rubber is formed. This rubber latex was tried last spring (1930) as a dressing for tree wounds. It was applied to cuts immediately after they were made, a single coat being flowed on freely with a paint brush. The material dried quickly and the resulting rubber film seemed relatively impervious, highly elastic, strongly adhesive to the wood, and of great tensile strength. Throughout the past growing season it has resisted the action of weather and the organic substances present on the wounds, and came through in excellent condition. In our experiments on about a dozen species of trees and, when applied under varied weather conditions, this dressing has not injured the cambium region nor retarded growth. In fact, by preventing desiccation of tissues, the production of callus has seemed to be somewhat stimulated as compared with that of

[1] This compound is sold under the name "Vultex" by the Vultex Corporation of America, 708 Main Street, Cambridge, Mass.

untreated wounds. Besides the excellent results given by the material, its cost is reasonable and it is easily applied with a brush or a sprayer without any previous treatment or heating. It may also be used when the temperature is below freezing. The lowest temperature at which it was used by the writer was 19° F. It was not affected by this temperature.

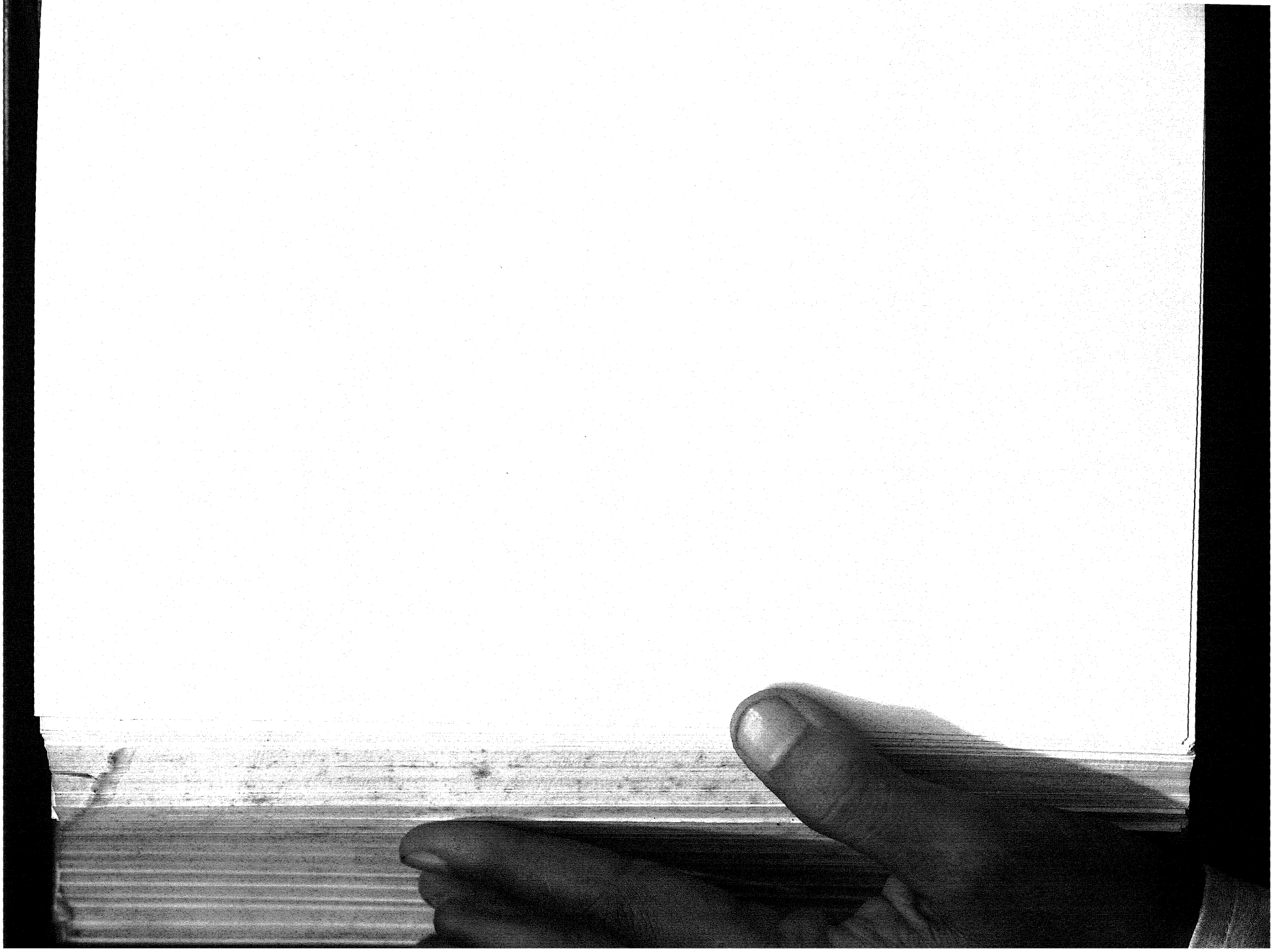
With the availability of a material so well suited to the task of ringing, it is probable that double dressing will become increasingly popular and possibly largely replace single dressings for use on large wounds. The writer does not seek to suggest that the use of this latex be restricted to the ringing of cuts. It already has given most excellent results in the treatment of small wounds. It has proved its merit, when promptly applied, for the dressing of large superficial wounds due to barking, a type of injury frequently inflicted on street trees by automobiles. It holds promise for any who might care to test its application to the various processes of budding and grafting.

This rubber compound warrants inclusion in the trials of all who are investigating the subject of wound dressings for trees. It justifies further experimentation with double, or ringed, dressings despite the increased cost and bother their use occasions. It is a material for which the research of the horticulturist, pathologist, and physiologist may find considerable application.—RUSH P. MARSHALL, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with Osborn Botanical Laboratory, Yale University.

Pseudomonas prunicola and Bacterium citriputeale.—The bacterial disease of stone fruit trees, caused by *Ps. prunicola,* recently described from Britain, has many of its cultural characteristics identical with those of citrus-blast or black-pit caused by *Bact. citriputeale.* The writer, through the courtesy of Dr. Wormald,[1] secured a culture of his Prunus organism, *Ps. prunicola.* Its growth on dextrose-potato agar resembles closely that of the citrus-blast organism, and inoculations by it through wounds in nearly ripe lemon fruits that were kept in a moist chamber produced within 10 days typical black pit-like lesions identical in appearance with those that have been produced with the citrus-blast, lilac, and Prunus organisms that have been isolated from California material.[2] It seems that *Ps. prunicola* is apparently very similar to, if not identical with, the lilac organism, *Bact. syringae,* with the citrus-blast organism, *Bact. citriputeale,* and with the Prunus organism, *Bact. cerasi,* as reported from Oregon and California.—CLAYTON O. SMITH, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[1] Wormald, H. Bacterial diseases of stone-fruit trees in Britain. II. Ann. Appl. Biol. 17: 725–744. 1930.

[2] Smith, Clayton O., and Howard S. Fawcett. A comparative study of the citrus blast bacterium and some other allied organisms. Jour. Agr. Res. 41: 233–246. 1930.



BOOK REVIEW

Sorauer, Paul. *Handbuch der Pflanzenkrankheiten, Band II, Fünfte Auflage.* Herausgegeben von Dr. O. Appel, Dr. Paul Graebner, und Dr. L. Reh. 758 pp. Paul Parey, Berlin. 1928.

As pointed out by Dr. Appel in the introduction to this volume, Sorauer's Handbuch has become not only one of the classics in plant pathology, but the successive editions mirror in a unique way the progress of the science. The preponderating emphasis on predisposition and non-parasitic diseases in the earlier editions and the increasing emphasis on parasitic diseases and pathogens in the later ones reflect to some extent the transition from the autogenic to the pathogenic theory of the cause of plant disease, although the influence of environment on the development of diseases is by no means neglected in the last edition. Indicative of the progress in knowledge is the fact that the first 295 pages of volume 2 of this 5th edition are devoted to bacterial diseases, whereas in the 4th edition only 86 were devoted to this subject.

The book is divided into two parts: the first deals with diseases caused by bacteria, and the second with those caused by Myxomycetes, Phycomycetes, and Ascomycetes.

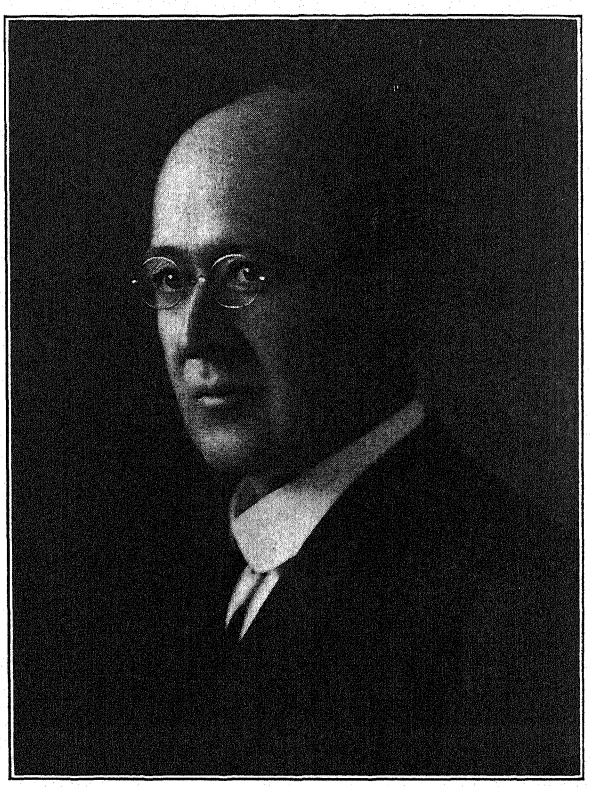
The section on bacterial diseases is thoroughly done. Following an introduction, in which certain generalities and principles are discussed, there is a classification of bacteria according to Migula's System. The diseases are then grouped according to orders of host plants. This arrangement has certain obvious advantages and disadvantages, although, in the case of bacterial diseases, the former outweigh the latter. The disease symptoms are thoroughly, sometimes almost minutely, described, the historical summaries are useful, the organisms are described in detail, and the pathological anatomy, contributing factors, and economic importance and control measures are discussed in some detail for the most important diseases. The section is an excellent compilation of our knowledge of bacterial diseases up to 1926; the important literature, for the most part, has been summarized and evaluated with judgment and skill. The references are given in footnotes, which are convenient in reading, but have the disadvantage of not presenting an easily available bibliography. The section is a very commendable and useful summary, written in a style that should not be difficult for English-speaking scientists with a fairly good knowledge of German. The principal criticism, in my opinion, is the lack of adequate headings. For example, one finds on page 121 the heavily leaded center head, "24. Bakteriosen der Rosaceen." The description of diseases of this group ex-

tends to page 159, but the absence of headings may prove inconvenient for a busy reader.

In Part II the diseases caused by slime molds, Phycomycetes, and Ascomycetes are discussed. The arrangement is according to the taxonomic position of the pathogens. There is a certain lack of uniformity in treatment and viewpoint, as would be expected from the fact that different sections were prepared by different contributors. In general, one is impressed by the comprehensiveness of mycological and pathological information presented, although in certain sections the mycological viewpoint seems to predominate over the pathological. This probably is almost unavoidable in a book of so wide a scope, where an attempt is made to mention, briefly at least, virtually all pathogens. The mycological proglems involved are, of course, also sometimes puzzling and the attempt to elucidate them is commendable, although the strictly pathological phases are only rather briefly discussed in a few instances. In general, the balance between various phases is good. The headings in Part II are helpful, and, for the most part, well arranged.

Mechanically, the book is good. It is printed on good paper; the type is clear; the illustrations are mostly good, although a few more, illustrating certain diseases, would be helpful; and typographical errors are no more numerous than would be expected.

The prodigious amount of work required in compiling a book of this kind would cause a critic to be lenient, even if there were more in the book to criticize adversely. Those responsible for the book deserve rich credit, not only for bringing together in one volume such a tremendous amount of widely scattered information but also for excellent perspective in the treatment of the material.—E. C. STAKMAN, University Farm, St. Paul, Minn.



WARNER JACKSON MORSE

PHYTOPATHOLOGY

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WARNER JACKSON MORSE
1872–1931

EDITH M. PATCH

Warner Jackson Morse came to the Maine Agricultural Experiment Station in 1906 to head the Department of Plant Pathology. In 1921 he became Director of the Experiment Station and he still held this title at the time of his death on the twenty-fifth of March, 1931.

During his quarter-of-a-century residence in Maine, I knew him as colleague, friend, and neighbor and, for the last decade, as an official superior. Because of this long acquaintance, I have been asked to write this sketch.

To speak appropriately of Doctor Morse is to speak informally and with simplicity. He drove one day, a few years ago, into the country cemetery across the river from his home. His glance sought the hills in the far distance, and he said to his wife, "This is good enough for me." It was fitting that his words were remembered and that, when the time came for them, his last rites were observed in the town of Orono, which had been his dwelling place for about twenty-five years.

Perhaps lack of ostentation is natural to the country bred. In keeping, certainly, with the associations of his early life, were his later occupations. This man was always at heart a farmer. The products of the soil interested him. The subjects that he taught, as a young man, were botany and bacteriology. Later, his research work was concerned with the diseases of plants to the end that the health of certain crops might be safeguarded. Obviously, his earnest interest in agricultural matters, from practical as well as from experimental aspects, contributed significantly to his economic service to Maine.

Outdoor interests were the background also of his recreational pursuits. He found keen pleasure in working in his vegetable and flower gardens. He made a squirrel welcome to picnic luncheons on the ledge of his office window. Bluebirds and tree swallows nested in boxes he made and placed for them on the trees about his home. He planted bushes that grosbeaks might enjoy their fruit. And, of all the books that he read during his long illness, perhaps none gave him greater enjoyment than "Jack Miner and the Birds."

Although interest and industry are necessary to the sort of success achieved by Doctor Morse, they are not in themselves sufficient. Tenacity of purpose, too, is a requisite; and this he had.

And, since he headed the Maine Agricultural Experiment Station during those most meager years immediately preceding the Purnell appropriations, his conservativeness stood the institution in good stead. Indeed conservatism might well be indicated as one of his outstanding qualities. The known and the tried made a much stronger appeal to him than the new and the untested. He never forgot an old friend. An interest account in a stable savings bank outweighed any temptations to invest in stocks and bonds. And these same tendencies, characteristic of his personal affairs, influenced his official attitude.

Thus, under his direction, new departments, new lines of research, new projects were ventured upon; but only when he became convinced of their importance and significance.

His conservatism, however, could not be interpreted as a concession in quality. For it was only the best that appealed to him—whether in the choice of tools and mechanical devices for his personal use or in sanctioning standards and ideals in his professional and official capacities.

In keeping with his other sturdy qualities was his unvarying honesty and indiscriminating justness. No one associated with him as colleague or subordinate had reason to feel unfairly treated. There was no department or investigator favored at the expense of another.

Warner Jackson Morse was born in Waterbury Center, Vermont, October 30, 1872. He was the son of Daniel Jackson and Jane (McKee) Morse. In 1898 he married Mary Leland, of Johnson, Vermont, who survives him, as does their daughter, Ruth Morse Burbank. Surviving him also are two sisters, Mrs. Ida Grout, of Waterbury, Vermont, and Mrs. Mae Heath, of Chautauqua, New York.

He was graduated from the Johnson (Vermont) Normal School in 1893. From the University of Vermont he received the earned degrees B.S. in 1898 and M.S. in 1903. The same institution bestowed upon him the honorary degree Sc.D. in 1923. He pursued his doctorate work at the University of Wisconsin, where he took his Ph.D. in 1912.

He was a teacher of natural sciences, Montpelier (Vermont) Seminary, 1899–1901. At the University of Vermont he was instructor of botany, 1901–1905, and assistant professor in bacteriology, 1905–1906. He was also assistant botanist at the Vermont Agricultural Experiment Station from 1901 until 1906, when he came to the sister institution in Maine.

He was a member of Sigma Xi, Phi Kappa Phi, Kappa Sigma, Alpha Zeta, The American Phytopathological Society (charter member), and the

Botanical Society of America, and fellow of the American Association for the Advancement of Science. He was a Mason.

His published papers total more than fifty titles.

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FURTHER STUDIES ON THE FUNGICIDAL EFFICIENCY OF CHEMICAL DUSTS CONTAINING FURFURAL DERIVATIVES

J. J. WILSON[1] AND C. S. REDDY[2]

INTRODUCTION

Organic-mercury compounds having a furan ring were found by the junior writer,[3] in 1927, to be applicable as dust fungicides for the control of the dry-rot seedling blights of corn. The favorable preliminary results led to a more intensive study to determine what methods of preparation and what concentrations of the organic-mercury compounds were most beneficial in the control of three seed-borne diseases of corn. Laboratory and field experiments were conducted in 1928 and 1929 for these purposes and to determine further the merits of certain laboratory biological tests by correlating laboratory and field results.

COMPOUNDS STUDIED

The structural formulae of these organic-mercury compounds having the furan ring are not yet fully understood. Their methods of preparation have been described in a previous paper by the junior writer.[4] For convenience in subsequent discussion they are divided into three groups.

(A) E2C. The reaction product formed as precipitate when a solution of mercuric chloride is stirred into a furfural solution previously subjected to the Cannizzaro reaction and to which ammonia has been added.

(B) E4. The reaction product formed as a precipitate when a solution of mercuric nitrate is stirred into a furfural solution previously subjected to the Cannizzaro reaction.

(C) G1. The reaction product formed as a precipitate when solutions of mercuric chloride and furfuramide are stirred together. Either alcohol, acetone, or aqueous solution of ammonium chloride is used as the solvent for the mercuric chloride and furfuramide.

The resultant precipitate of each of the three groups was dried, finely ground, and mixed with talc as a filler. In this manner a number of fungi-

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[3] Reddy, C. S. Fungicidal efficiency of chemical dusts containing furfural derivatives. Phytopath. 20: 147–168. 1930.

[4] *Loc. cit.*

cidal dusts having different concentrations of the toxic agents were prepared. These experimental dusts are designated by numbers and the nature of the organic-mercury precipitate may be identified by the symbol E2C, G1, or E4.

In order to find out whether or not these fungicides could be manufactured without difficulty or excessive cost, all of the E2C and G1 dusts, used in 1928, were prepared by the Miner Laboratories, Chicago, Illinois.

LABORATORY EXPERIMENTS—1928

The number of fungicidal dusts prepared was much larger than could be given field experimentation and had to be reduced on the basis of data secured from laboratory experiments. The importance of these preliminary tests was plainly evident. Unless there was a high correlation between the laboratory and field results, the most beneficial dust fungicide might be discarded without field trial. Previous experiments had shown that the visible-root sand-culture method devised by Raleigh[5, 6] had merit in selecting dusts that combined high fungicidal value with little or no injurious effect upon living corn-plant tissues. This method was used in these experiments and later was varied to overcome some of the difficulties of its manipulation. The variation consisted in the use of dairy cups in place of the crystallization dishes. This permitted any slight excess of water to drain through holes punched in the bottom of the cups. Also, when dairy cups were used the seed was placed midway rather than on the bottom, where it was thought soluble toxic agents might accumulate. Wherever this preliminary method is used, it is designated the dairy-cup sand-culture method.

A detailed study of the E2C, G1, and E4 compounds required that variations in the method of preparation and in the amount of the organic-mercury precipitates be made and tested.

One hundred and forty kernel lots of a standard variety of dent corn known to be highly infected with *Diplodia zeae* (Schw.) Lev. were treated with each of 12 E2C, 43 G1, and 2 E4 dusts. Seven replications of 20 kernels of each seed-treatment lot were planted in crystallization dishes. These dishes were 10 cm. in diameter and 5 cm. deep. Washed river sand was used to cover the corn to a depth of 4 cm. The cultures were held at 18° to 22° C. and the soil moisture was maintained near 75 per cent of saturation.

After 12 days' growth, the plants were washed free from sand and weighed, and the growing plants and lesions caused by Diplodia were

[5] Raleigh, W. P. A preliminary method of measuring the relative efficiency of seed corn disinfectants. (Abst.) Phytopath. 18: 140. 1928.

[6] Infection studies of *Diplodia zeae* (Schw.) Lev. and control of seedling blights of corn. Iowa Agr. Exp. Sta. Res. Bul. 124. 1930.

TABLE 1.—*Laboratory experiments on the fungicidal action of different concentrations of E2C dusts on seed corn infected with Diplodia zeae, Ames, Iowa, 1928*

Dust No.	Constituent parts			Temperature precipitate dried	Growing plants	Green weight	Relative value	Plants with lesions
	Furfural	Mercuric chloride	Talc					
				°C.	*Number*	*Grams*		*Per cent*
1	5	6.6	50	20 to 24	110	171	266	12.7
2	"	"	60	"	120	186	289	25.8
3	"	"	75	"	124	196	310	18.5
4	"	"	100	"	125	194	301	18.4
5	"	"	150	"	123	189	290	21.9
6	"	"	300	"	121	196	317	16.5
7	"	"	50	90	127	198	309	4.7
8	"	"	60	"	131	206	324	9.9
9	"	"	75	"	131	195	290	3.0
10	"	"	100	"	127	179	252	12.5
11	"	"	150	"	127	203	324	6.2
12	"	"	300	"	127	199	312	9.4
Check[a] (not treated)					120	172	247	44.2

[a] Computed from 27 replications.

counted in each treatment and check. From these data the average green weight per plant and the percentage of plants with lesions were computed for each seed treatment. An arbitrary method of expressing the relative value of each treatment was obtained by squaring the total green weight and dividing by the number of growing plants. In this way the value was determined by a combination of individual and total plant weights.

The interpretation of the biological response in such laboratory tests involves a study of the fungicidal effectiveness of the dust and the physiological action of the treatment on the growth of the corn seedling. Care was taken in the study of each individual dust to determine whether or not depressing effects were produced on plant growth. In measuring the fungicidal efficiency of the dusts, which were selected to be used in the field trials, low lesion counts were not considered sufficient, but were used in conjunction with green weight and number of living plants.

Table 1 shows the results obtained in laboratory trials with 12 E2C dusts. The treated seeds produced in every instance a lower percentage of plants with lesions and a higher relative value for total green weight than seed not treated. The data also show that the plants from seeds treated with E2C dusts dried at 20° to 24° C. had higher lesion counts than those treated with E2C dusts dried at 90° C. This probably was due to the more resinous nature of the E2C precipitate when dried at the lower temperature. Resinous precipitates are often difficult to grind and mix with talc and such difficulties may cause dust fungicides to have poor physical properties, resulting in a reduction of fungicidal efficiency. E2C Nos. 8 and 11 were selected for field trials. These 2 dusts had equal relative values and showed low percentages of plants with lesions.

Five different G1 precipitates were made by varying the proportions of furfuramide and mercuric chloride and by using different solvents. From these precipitates 43 different dust fungicides were made by varying the amount of talc filler.

Laboratory experimental results of these 43 G1 dusts, as shown in tables 2 and 3, indicate that the variation in the quantity of furfuramide or mercuric chloride produced no marked effect. There were, however, differences in the resinous character of the resultant precipitates, which had to be taken into consideration in the final selection of the dusts. The lower concentrations of the G1 precipitate gave poorer control of the disease and, therefore, were associated with higher lesion counts. Without exception, all concentrations of the G1 precipitate had some fungicidal value because the percentage of plants with disease lesions was lower and the relative value higher in all of the treatments than in the checks not treated.

On the basis of the data presented in tables 2 and 3, G1 dusts Nos. 13, 50, 51, 53, and 61 were selected for field trial. Number 13 contained

TABLE 2.—*Laboratory experiments on the fungicidal action of different concentrations of G1 dusts on seed corn infected with Diplodia zeae, Ames, Iowa, 1928*

Dust No.	Constituent parts			Temperature precipitate dried	Growing plants	Green weight	Relative value	Plants with lesions
	Furfuramide	Mercuric chloride	Talc					
				°C.	*Number*	*Grams*		*Per cent*
13	5	6	50	20 to 24	125	206	339	0.0
14	"	"	60	"	130	203	317	0.7
15	"	"	75	"	127	199	312	6.2
16	"	"	100	"	126	197	308	6.3
17	"	"	150	"	125	196	307	6.4
18	"	"	50	90	129	206	329	2.3
19	"	"	60	"	121	195	314	4.1
20	"	"	75	"	130	202	314	1.5
21	"	"	100	"	127	196	304	14.1
22	"	"	150	"	134	198	316	4.0
23	7	3.5	50	20 to 24	129	206	329	3.1
24	"	"	60	"	130	188	272	6.9
25	"	"	75	"	121	187	289	8.2
26	"	"	100	"	132	203	315	8.3
27	"	"	150	"	134	206	317	18.6
28	"	"	50	90	126	192	292	3.9
29	"	"	60	"	122	183	274	5.7
30	"	"	75	"	130	210	339	5.3
31	"	"	100	"	117	187	299	17.9
32	"	"	150	"	121	181	271	20.6
33	3.5	7	50	20 to 24	122	193	308	9.8
34	"	"	60	"	131	207	317	3.8
35	"	"	75	"	125	205	336	9.6
36	"	"	100	"	124	203	334	3.2
37	"	"	150	"	123	189	290	9.7
38	"	"	50	90	120	191	304	2.5
39	"	"	60	"	129	202	314	3.1
40	"	"	75	"	124	194	303	3.2
41	"	"	100	"	125	210	353	11.2
42	"	"	150	"	132	197	295	12.8
61	5	15	180	"	122	201	331	4.9
Check[a] (not treated)					120	172	247	44.2

[a] Computed from 27 replications.

TABLE 3.—*Laboratory experiments on the fungicidal action of different concentrations of G1 dusts (ammonium chloride solvent) used on seed corn infected with Diplodia zeae, Ames, Iowa, 1928*

Dust No.	Constituent parts					Temperature precipitate dried	Growing plants	Green weight	Relative value	Plants with lesions
	Furfuramide	Mercuric chloride	Water	Ammonium chloride	Talc					
						°C.	*Number*	*Grams*		*Per cent*
43	15	5	500	30	50	20 to 24	127	195	299	7.8
44	"	"	"	"	100	"	127	196	302	2.3
45	"	"	"	"	150	"	126	188	280	11.9
46	"	"	"	"	200	"	126	202	325	7.1
47	"	"	"	"	250	"	127	210	347	11.8
48	"	"	"	"	300	"	132	211	337	13.6
49	"	"	"	"	50	90	122	189	293	2.4
50	"	"	"	"	100	"	132	218	360	3.7
51	"	"	"	"	150	"	130	208	333	7.6
52	"	"	"	"	200	"	132	199	300	6.8
53	"	"	"	"	250	"	122	197	317	10.6
54	"	"	"	"	300	"	199	190	303	24.3
Check[a] (not treated)							120	172	247	44.2

[a] Computed from 27 replications.

TABLE 4.—*Summary of laboratory data on the 14 fungicidal dusts selected for field experiments. Ames, Iowa, 1928*

Dust No.	Identification symbol or name	Constituent parts					Solvents other than water	Relative value	Comparison with highest relative value
		Furfural	Furfuramide	Mercuric chloride	Mercuric nitrate	Talc			
									Per cent
8	E2C	5		6.6		60	none	324	90
11	E2C	5		6.6		150	none	324	90
13	G1		5	6.0		50	$(CH_3)_2CO$	340	94
50	G1		15	5.0		100	NH_4CL	360	100
51	G1		15	5.0		150	NH_4CL	333	92
53	G1		15	5.0		250	NH_4CL	316	87
58	E4	30			5	95	none	359	99
61	G1		5	15.0		95	$(CH_3)_2CO$	331	91
61a	G1		5	15.0		93	$(CH_3)_2CO$		
61b	G1		5	15.0		97	$(CH_3)_2CO$		
67	F–13							317	88
63	Bayer dust							301	83
64	Semesan Jr.							328	91
65	Merko[a]							269	74
Check (not treated)								247	69

[a] The low relative value of Merko in comparison with the other commercial dusts is probably due to overwatering two cultures in the Merko series.

approximately the same concentration of toxic agent as the G1 dust used in 1927. Numbers 50, 51, and 53 represent different concentrations of G1 prepared by using ammonium chloride as the solvent. Number 61 was chosen because the precipitate was not resinous and could be easily ground and mixed with a talc filler. Three different concentrations of No. 61 were made up in large quantities for field trials. The concentrations were made to approximate as closely as possible dusts Nos. 30, 36, and 41. These last 3 dusts had high relative values but were not easily prepared because of difficulty in grinding the precipitate.

The laboratory data on 14 fungicidal dusts selected for 1928 field experimental study are summarized in table 4.

LABORATORY EXPERIMENTS—1929

In 1929 a study was made of the relative fungicidal efficiency of G1 in concentrations of 3, 4, and 5 per cent of the toxic agent, mercury furfuramide. G1 dusts of these three strengths were compared with Merko in

TABLE 5.—*Laboratory data on emergence, disease lesions, and relative-weight value obtained by treating 2 lots of Diplodia-infected seed corn with dust Nos. 61, 61b, Sterocide, and Merko. Ames, Iowa, 1929*

Dust No.	Growing plants	Green plant weight	Relative value	Comparison with highest relative value	Plants with lesions
	1928 Diplodia-infected seed				
	Number	*grams*		*Per cent*	*Per cent*
(G1–5%)					
61	79	165	344	100	13.3
(G1–3%)					
61b	77	162	341	99	15.0
(G1–4%)[a]	78	159	324	94	12.2
Merko	78	160	328	95	8.0
Check (not treated)	65	125	238	69	35.0
	1927 Diplodia-infected seed				
(G1–5%)					
61	90	179	356	92	1.1
(G1–3%)					
61b	87	175	352	91	4.5
(G1–4%)[a]	88	176	352	91	2.0
Merko	87	184	385	100	0.0
Check (not treated)	86	159.5	294	76	13.8

[a] Sterocide Average of 9 lots.

laboratory trials on 2 different lots of Diplodia-infected seed corn. The dairy-cup sand-culture method was used in making the laboratory biologic tests. Six replications of 15 kernels were planted from both seed lots after being treated by the 4 dusts. The 3 and 5 per cent concentrations of G1 were dusts Nos. 61 and 61b of the 1928 experimental series. The 4 per cent concentration of G1 is the commercial form placed on the market in 1929 and known by the trade name, Sterocide. The laboratory data from 9 individual lots of Sterocide (4 per cent toxic agent) were averaged, and these data are compared in table 5 with data from dust No. 61 (5 per cent toxic agent), No. 61b (3 per cent toxic agent), and Merko.

All 4 dust seed treatments (data in table 5) produced a decided increase in the weight of the plants and a reduction in the percentage of plants with lesions. Seed treated with dust No. 61b (3 per cent toxic agent) produced a higher percentage of plants with lesions than seed treated with No. 61 (5 per cent toxic agent) or Sterocide (4 per cent toxic agent). The data indicated that 61b was possibly a little too weak in fungicidal action.

FIELD EXPERIMENTS

In order to determine the reliability of the laboratory methods used in measuring the fungicidal efficiency of seed-corn dust fungicides, field trials were conducted with the dusts selected in the 1928 and 1929 laboratory experiments.

Field experiments—1928. The 10 best E2C, G1, and E4 dusts selected in laboratory trials and 3 commercial dusts (summary of results in table 4) were used in seed-treatment experiments on 2 different lots of diseased seed corn and a lot of nearly disease-free seed corn. All 3 lots of seed corn were selected by an individual ear sand-tray-germinator test of a high-yielding local strain of Reids Yellow Dent Corn. One of the 2 lots of diseased seed corn was infected with *Diplodia zeae* and was selected from ears showing large infected zones with viable kernels. The other lot of diseased seed was infected with *Basisporium gallarum* Moll. and was selected from ears having viable kernels with adhering Basisporium spores. The nearly disease-free seed was secured from sound ears that were germinator-selected to eliminate practically all diseased kernels.

The diseased seed corn was used to measure the relative fungicidal efficiency of the different dusts and the nearly disease-free seed corn was used to detect any injurious effect caused by the different dusts on the growth of the plants. Field stand and acre yield were used to measure these two factors.

Thirty hill plots, replicated 10 times, were used for testing each dust treatment and repeated for each of the 3 different seed lots. Seed not treated was included as checks in each replication. The rate of planting

was 2 kernels per hill in all plots except those planted with Basisporium-infected seed in which the rate was 3 kernels per hill. The kernels for each hill were counted and planted by hand.

The plots were planted May 5 in order to subject the seed to any possible cold weather and unfavorable growing conditions that might accompany a moderately early planting. This aim, however, was not realized because the season of 1928 proved unusually mild in temperature. The seed germinated quickly and the plants made a rapid, continued growth. The seed-treatment plots were cultivated throughout the season in the same manner as a regular field of corn. After the first and second cultivation, all plants covered by the plow were uncovered to prevent a mechanical loss in stand.

The effect of different dusts on stand. The field-stand data presented in table 6 were taken 3 weeks after planting. On nearly disease-free seed,

TABLE 6.—*Field stands from nearly disease-free, Basisporium-infected, and Diplodia-infected seed corn not treated and treated with 10 dusts containing organic mercury with a furan ring and three commercial dusts. Ames, Iowa, 1928*

Dust No.	Nearly disease-free seed		Basisporium-infected seed		Diplodia-infected seed	
	Stand	Increase or decrease	Stand	Increase or decrease	Stand	Increase or decrease
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Check (not treated)	91.67		70.67		62.67	
8 (E2C)	89.26	−2.63	70.00	− .73	68.00	+ 5.81
11 (E2C)	92.04	+ .40	71.89	+1.33	70.84	+ 8.90
13 (G1)	87.50	−4.55	72.34	+1.82	76.00	+14.53
61a (G1)	90.39	−1.40	71.67	+1.09	72.84	+11.80
61 (G1)	91.17	− .54	74.45	+4.12	69.84	+ 7.81
61b (G1)	89.84	−1.99	73.23	+2.79	74.00	+12.35
65 (Merko)	91.67	−1.63	70.34	− .36	76.00	+14.53
67 (F13)	90.34	−1.45	74.78	+4.48	70.50	+ 8.54
50 (G13)	90.34	−1.45	74.89	+4.60	76.50	+15.75
51 (G13)	92.17	+ .54	75.56	+5.33	70.50	+ 8.54
53 (G13)	90.50	−1.28	73.56	+3.15	73.67	+11.99
58 (E4)	91.34	− .37	74.12	+3.76	74.84	+13.26
63 (Semesan Jr.)	91.00	− .73	73.34	+2.91	73.67	+11.99
64 (Bayer Dust)	91.00	− .73	71.56	+ .97	70.34	+ 8.36

2 treatments, Nos. 8 and 13, reduced the stand sufficiently to indicate injury. Both dusts contained a high concentration of the active precipitate. Dust No. 8 reduced the stand 2.63 per cent, and No. 13, 4.55 per cent. Percentage increases or decreases in the other 12 dust treatments were relatively small. Dust treatments of Basisporium-infected seed with Nos. 67, 50, and 51

increased the stand more than 4 per cent and treatments with Nos. 61 and 58 were nearly as efficient in increasing the stand.

Significant increases in stand were secured by treatment of Diplodia-infected seed corn. The treated seed produced 5.81 to 15.75 per cent more plants than the checks (not treated).

Under these favorable conditions for corn growth, such increases in the percentage of stand from seed treatment are secured only on badly diseased seed. Continued unfavorable weather, at the beginning of seed germination, usually results in much larger increases in stands.

Yields obtained. The plots were harvested during the last week of October. The corn was exceptionally well matured and dry. Acre yields of ear corn (75 lbs. per bu.) from the 3 different seed lots were computed for 10 replications of each treatment and check.

Table 7 shows that treatments of nearly disease-free seed corn produced

TABLE 7.—*Acre yields of field plots planted with nearly disease-free seed corn treated with 10 dusts having E2C, G1, and E4 as their toxic agents and 4 commercial dusts. Ames, Iowa, 1928*

Dust No.	Acre yield		Increase or decrease	Odds[a]
	Not treated	Treated		
			Bushels	
8 (E2C)	69.2	69.8	+0.6	1:1
11 (E2C)	69.2	70.4	+1.2	1:1
13 (G1)	69.2	68.8	−0.4	1:1
61a (G1)	68.4	70.7	+2.3	6:1
61 (G1)	68.4	71.6	+3.2	49:1
61b (G1)	68.4	71.0	+2.6	6:1
65 (Merko)	67.7	68.1	+0.4	1:1
67 (F13)	67.7	71.0	+3.3	252:1
50 (G1)	67.7	70.6	+2.9	11:1
51 (G1)	67.7	70.0	+2.3	29:1
53 (G1)	68.8	69.0	+0.2	1:1
58 (E4)	68.8	70.5	+1.7	4:1
63 (Semesan Jr.)	68.8	72.4	+3.6	171:1
64 (Bayer Dust)	70.1	71.1	+1.0	1:1

[a] Student's method.

no outstanding increases in yield. Dusts Nos. 67, 63, and 61 increased the yield slightly over 3 bushels and the odds were significant. Only 1 dust, No. 13, produced any decrease in yield and this was not significant. (Odds 1:1.) These data indicate that no injury has been caused by seed treatment.

The yield data from the field plot planted with Basisporium-infected seed are presented in table 8. It will be seen that no significant odds were

TABLE 8.—*Acre yields of field plots planted with Basisporium-infected seed corn treated with 10 dusts having E2C, G1, and E4 as their toxic agents and 4 commercial dusts. Ames, Iowa, 1928*

Dust No.	Acre yield		Increase or decrease	Odds[a]
	Not treated	Treated		
			Bushels	
8 (E2C)	79.6	74.1	−5.5	9:1
11 (E2C)	79.6	78.2	−1.4	2:1
13 (G1)	78.3	79.4	+1.1	2:1
61a (G1)	78.3	79.6	+1.3	2:1
61 (G1)	78.3	80.2	+1.9	5:1
61b (G1)	77.1	81.6	+4.5	11:1
65 (Merko)	77.1	79.0	+1.9	4:1
67 (F13)	77.1	78.5	+1.4	2:1
50 (G1)	77.1	79.4	+2.3	5:1
51 (G1)	79.5	82.9	+3.4	6:1
53 (G1)	79.5	79.7	+0.2	1:1
58 (E4)	79.5	81.5	+2.0	8:1
63 (Semesan Jr.)	81.6	78.0	−3.6	15:1
64 (Bayer Dust)	81.6	82.0	+0.4	1:1

[a] Student's method.

secured in the increase in yield from seed treatment of Basisporium-infected seed corn. The greatest increases in yield were secured with dusts Nos. 61b and 51. Both dusts contained a low percentage of organic mercury. Dusts Nos. 8, 11, and 63, each of which contained a high percentage of organic mercury, decreased the yields.

The yield from the field plot planted with Diplodia-infected seed are presented in table 9 in which it is shown that outstanding increases in yield resulted from treatments of Diplodia-infected seed corn. Dust No. 65 increased the yield 15.1 bu. per acre. It was nearly equaled by dust No. 50, which increased the yield 14.4 bu. per acre. Dusts Nos 65 and 50 gave odds of 4,999 to 1. No. 61 increased the yield only 2.5 bushels, but 61a and 61b increased yields more than 11 bu. per acre. This seems to show that the relative rank of No. 61 was below its deserved position.

These results indicate that a fungicidal efficiency of practical value is present in the dusts containing E2C, G1, and E4 precipitates.

In order to compare the dusts containing the furan ring with the other dusts (3 of the 4 being commercial dusts), the dust treatments were ranked on the basis of increases in acre yield in each of the 3 field plots. It was found that seed treatments containing the furan ring occurred twice in the 4 highest in 1 experiment (Table 7), 4 times out of 4 in another (Table 8), and 3 out of the 4 times in the other field experiment (Table 9).

TABLE 9.—*Acre yields of field plots planted with Diplodia-infected seed corn treated with 10 dusts having E2C, G1, and E4 as their toxic agents and 4 commercial dusts. Ames, Iowa, 1928*

Dust No.	Acre yield		Increase or decrease	Odds[a]
	Not treated	Treated		
			Bushels	
8 (E2C)	52.6	57.8	+ 5.2	15: 1
11 (E2C)	52.6	65.0	+12.4	29: 1
13 (G1)	54.6	66.3	+11.7	1110: 1
61a (G1)	54.6	66.0	+11.4	49: 1
61 (G1)	54.6	57.1	+ 2.5	4: 1
61b (G1)	52.0	63.7	+11.7	216: 1
65 (Merko)	52.0	67.1	+15.1	4999: 1
67 (F13)	52.0	61.9	+ 9.9	216: 1
50 (G1)	52.0	66.4	+14.4	4999: 1
51 (G1)	54.5	62.0	+ 7.5	61: 1
53 (G1)	54.5	63.3	+ 8.8	262: 1
58 (E4)	54.5	64.2	+ 9.7	100: 1
63 (Semesan Jr.)	57.1	67.6	+ 9.5	54: 1
64 (Bayer Dust)	57.1	61.4	+ 4.3	5: 1

[a] Student's method.

Field experiments—1929. The same concentrations of G1 studied in the laboratory experiments, namely, 3, 4, and 5 per cent of mercury furfuramide, were tested for fungicidal efficiency in field plots. One lot of seed corn infected with *Gibberella saubinetti* (Mont.) Sacc. and the other infected with *Diplodia zeae* were used in seed-treatment experiments with dusts Nos. 61 (G1–5 per cent), 61b (G1–3 per cent), Sterocide (G1–4 per cent), and Merko.

Six replications of 30-hill plots were planted on May 4 with seed corn treated with each dust. The method of planting was similar to that used in the 1928 field trials except that all plots were planted 2 kernels per hill.

Effect on stand. The season was less favorable for corn growth than in 1928, as was shown by the lower percentages of living plants in the 1929 plots.

The field stands following seed treatments with dusts Nos. 61, 61b, Sterocide, and Merko upon Diplodia-infected seed corn and Gibberella-infected seed corn are presented in table 10.

Diplodia-infected seed corn treated with dust No. 61b (G1–3 per cent) has the lowest percentage of stand. The plants in the laboratory trials treated with this same concentration of G1 also had the highest number of lesions. In the same experiments dust No. 61 (G1–5 per cent) consistently

TABLE 10.—*Field stands from Diplodia-infected and Gibberella-infected seed corn treated with dust Nos. 61, 61b, Sterocide, and Merko, Ames, Iowa, 1929*

Dust No.	Diplodia-infected seed		Gibberella-infected seed	
	Stand	Increase	Stand	Increase
	Per cent	*Per cent*	*Per cent*	*Per cent*
(G1–5%) 61	55.7	52.2	82.5	20.4
(G1–3%) 61b	49.8	36.1	74.3	8.5
Sterocide (G1–4%)	50.3	37.4	81.6	19.1
Merko	55.5	51.7	74.0	8.0
Check (not treated)	36.6		68.5	

increased field stands from both Diplodia and Gibberella-infected seed more than 61b (G1–3 per cent) or Sterocide (G1–4 per cent).

This would indicate that the 5 per cent concentration is more efficient as a fungicide than either of the 2 lower concentrations.

Yields obtained. As compared with nontreated seed, treatments with dust No. 61 (G1–5 per cent) and Merko increased the yields from Diplodia-infected seed corn more than 50 per cent (Table 11). Treatments with

TABLE 11.—*Acre yields obtained by treating Diplodia-infected and Gibberella-infected seed corn with dusts Nos. 61, 61b, Sterocide, and Merko, Ames, Iowa, 1929*

Dust No.	Diplodia-infected seed			Gibberella-infected seed		
	Acre yield	Increase	Percentage	Acre yield	Increase	Percentage
	Bushels	*Bushels*		*Bushels*	*Bushels*	
(G1–5%) 61	60.6	21.0	53.0	86.8	11.5	15.2
(G1–3%) 61b	54.5	14.9	37.6	85.0	9.7	12.9
(G1–4%) Sterocide	54.7	15.1	38.1	84.3	9.1	12.1
Merko	61.0	21.4	54.0	84.3	9.0	12.0
Check (not treated)	39.6			75.2		

dust No. 61b (G1–3 per cent) and Sterocide (G1–4 per cent) increased the yields 37.6 and 38.1 per cent, respectively.

A 15.3 per cent increase in yield was secured when Gibberella-infected seed corn was treated with dust No. 61 (G–5 per cent). The effects of the other 3 dusts were much alike, all producing about equal increases in yield.

SUMMARY

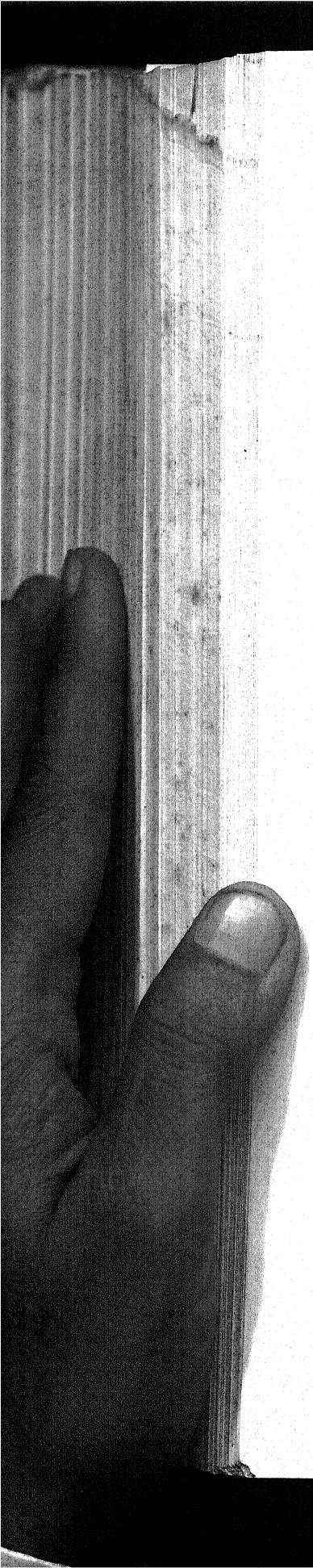
The scope of these investigations embraced the synthesis of a new toxic agent, the determination of the proportion of toxic agent to inert matter in the making of a fungicidal dust for seed-corn treatments, and the adjustment of the laboratory methods to commercial manufacture of the product.

A high correlation was found between the expressed fungicidal efficiency measured by laboratory biological tests and the stand and yield per acre secured by field experiments, using the same dust treatments.

Results of these experiments show that laboratory biological tests have been perfected that can be used to measure the relative fungicidal efficiency of seed treatments for corn.

From among 58 different concentrations of the organic-mercury compounds containing a furan ring, a concentration of 5 parts of G1 and 95 parts of talc was found to be the most consistent in giving the best fungicidal action on certain pathogenic organisms, such as *Diplodia zeae, Basisporium gallarum,* and *Gibberella saubinetii* without injurious effects on nearly disease-free seed.

When the fungicidal dusts were ranked on the basis of acre-yield increases in each of 3 large field experiments in 1928, it was found that treatments containing the furan ring occurred twice in the 4 highest in 1 experiment (Table 7), 4 times out of the 4 in another (Table 8), and 3 times out of the 4 in the other field experiment (Table 9).



PRELIMINARY OBSERVATIONS ON TWO SPECIES OF BEAUVERIA ATTACKING THE CORN BORER, PYRAUSTA NUBILALIS HÜBNER

C. L. LEFEBVRE[1]

INTRODUCTION

The purpose of this investigation was to note the morphological and physiological differences, if any, between certain species of Beauveria that attack the corn borer. One species of this fungus, occurring on the corn borer imported from Manchuria, killed from 80 to 90 per cent of the larvae, when these were subjected to conditions favorable for hastening the emergence of insect parasites for biological study. Large numbers of corn-borer larvae are imported annually from various countries by Government investigators interested in the control of this insect. By so doing they have established insect parasites in sufficient quantities to be of significance in the control of this pest in the United States. A somewhat similar species of this fungus has been reported on the chinch bug (2), the gipsy moth, and the satin moth, in the United States. The question therefore arises whether that species, first found parasitic on the corn borer in the European Corn Borer Laboratory at Arlington, Mass., is the same as the one already found on the chinch bug and other insects of this country. If so, why has it never heretofore been reported to occur on the corn borer, now so common in the United States? With the aim of casting light on this question the investigation was undertaken.

TAXONOMIC CONSIDERATIONS

The genus Beauveria in which these species belong is included in the tribe Verticillieae of the *Fungi Imperfecti*. It might be well at this time to state briefly some of the opinions and confused statements brought forward by different writers when referring to various representatives of this genus. For example, European writers have been using the generic names Botrytis Mich. and Sporotrichum Link when referring to members of this genus. Due to the confusion in naming members of these two groups, Vuillemin (10), 1912, instituted for them the new genus Beauveria; thus *Botrytis Bassiana* Bals. becomes *Beauveria Bassiana* (Bals.) Vuill. In

[1] The writer wishes to express his appreciation and gratitude to D. W. Jones, who has given him the opportunity to collaborate with Dr. Bartlett in this investigation.

He also wishes to acknowledge his indebtedness for helpful criticisms and suggestions to Professor William H. Weston, Jr., of Harvard University, under whom this work in its mycological aspects was carried on; and to Dr. W. H. Sawyer and Dr. M. T. Smulyan for cultures of *Beauveria globulifera* and *Isaria farinosa* (Dicks.) Fr., which they kindly furnished for comparative study.

1914 Picard (7) published a paper on entomogenous fungi in which he transferred Spegazzini's species *Sporotrichum globuliferum* to Vuillemin's genus, as *Beauveria globulifera.* From time to time others, chiefly Petch (5), Arnaud (1), and Billings and Glenn (2), have added considerable to our knowledge of these forms. This paper will be confined to a discussion of the two first-mentioned species.

The morphological differences between the species of Beauveria that are parasitic on insects are very slight, according to published reports by Petch and others. In the eight species of Beauveria described the main criteria used in separating them are the shape and size of spores, which are figured as being either oval or globose but may vary from these distinct extremes if the several species are grown on artificial media. Petch (5) makes clear this point in that, for *Beauveria Bassiana,* de Bary has described the conidia as being globular, 2.5 to 2.8 μ in diameter, while Delacroix records 2 to 2.5 μ as their dimensions, and Sawada has figured them as globose or broadly oval. Petch's own measurements, made from a culture secured from The National Collection of Type Cultures, records the conidia as being either broadly oval, 1.5 to 2.5 μ by 1.2 to 2 μ, or globose, 1.5 μ in diameter. Measurements of 250 conidia are summarized by the writer in table 4. In general, these measurements agree with those of Petch but, as they are tabulated in 0.5 μ classes and these, in turn, plotted on a curve of frequency distribution, the ranges in variation are more clearly brought out.

Since there is such a close similarity in spore size and shape between *Beauveria Bassiana* and *B. globulifera,* obviously other methods of separating these two organisms must be considered. Therefore, a more detailed study of the cultural characteristics on various artificial media and their virulence on the corn borer as a host was made by the writer, and the results are presented in this paper.

MATERIAL AND METHODS

The fungus, *Beauveria Bassiana,* was isolated from corn-borer larvae obtained from Dr. K. Bartlett, of the Corn Borer Laboratory, Arlington, Mass. For comparative study, cultures of *B. Bassiana* and *B. globulifera* were secured from the following sources: a culture of *B. Bassiana,* isolated by Dr. T. Petch, was obtained from the *Centralbureau voor Schimmelculture,* Baarn, Holland, and cultures of *B. globulifera* were obtained from Dr. W. H. Sawyer, Jr., and Dr. M. T. Smulyan, of the Gipsy Moth Laboratory, Melrose, Mass. Since these fungi grow very rapidly on potato-dextrose agar, it was relatively easy to secure and maintain a plentiful supply of inoculum.

In order to test the pathogenicity of *Beauveria Bassiana* and *B. globulifera* seven inoculation experiments were made during the course of the

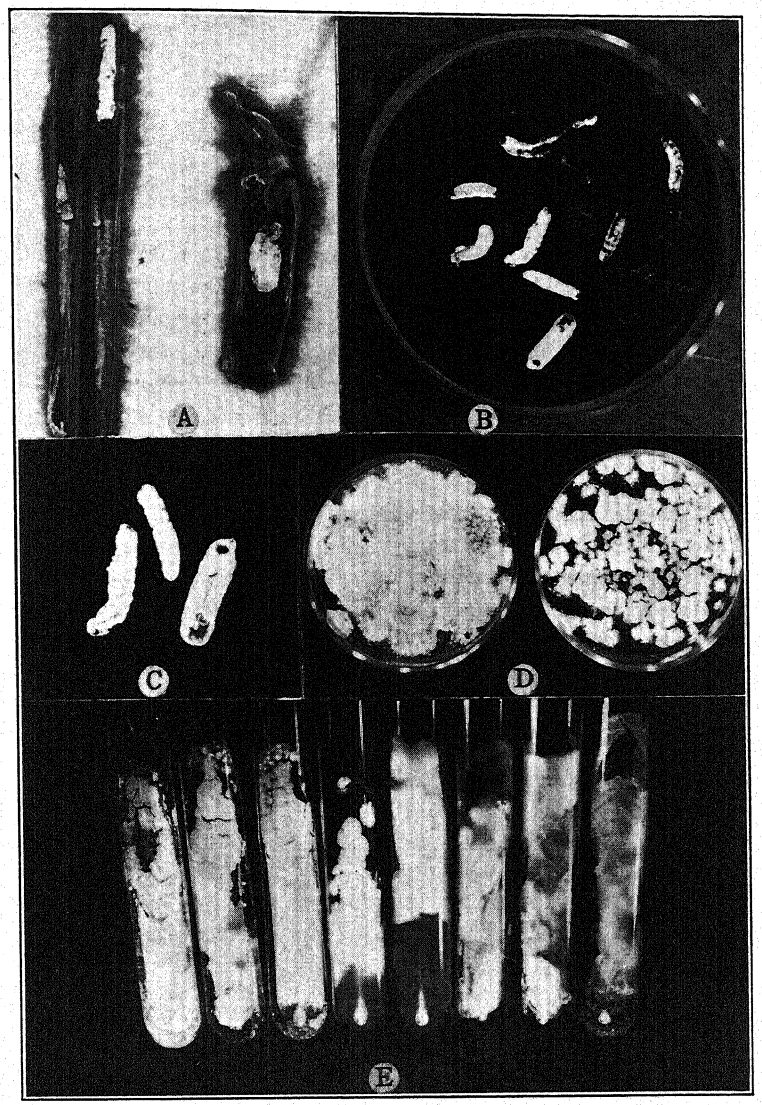


FIG. 1, A. Longitudinal sections of sweet-corn stems showing dead corn-borer larvae that were infected by dusting spores of *Beauveria Bassiana* on plants in the field. ×1. B. Corn-borer larvae killed within 2 days, after dusting with spores of *B. Bassiana,* in a moist chamber. ×⅔. C. Three dead corn borers showing larvae covered with white, mealy mycelium and spore mass of *B. Bassiana.* App. ×1. D. Left, *B. globulifera* on potato-dextrose agar showing elevated, cottony type of growth; right, *B. Bassiana* on potato-dextrose agar showing flat, mealy type of growth. ×½. E. Cultures of the two species on potato-dextrose agar, the left four showing the flat, mealy growth of *B. Bassiana;* the right four showing the elevated, floccose growth of *B. globulifera.* ×⅔.

summer, using as a host corn-borer larvae obtained through the courtesy of Dr. Bartlett. In each case a like number of larvae were used as controls.

Since, in the literature, there are accounts of the destructiveness of *Isaria farinosa* to various insects, it seemed desirable to test, along with these experiments, the possible pathogenicity of this fungus on the corn borer; therefore, comparative inoculation trials were made with this organism (Table 3).

Pure cultures of the fungus from infected corn borers are relatively easily secured after the larvae have been kept at room temperature for several days. During the course of this time a white mycelial outgrowth becomes evident, which turns later to a cream-colored, chalky, pulverulent mass of spores completely covering the larvae (Fig. 1, A and B). In this stage, by touching a sterile needle to this spore mass, a pure culture is readily isolated, or when isolating *Beauveria Bassiana* from corn-borer larvae collected in the field (Fig. 1, A) a minute bit of the fungus is easily scraped from the infected surface into tubes of melted potato agar, and these are poured into Petri plates, giving isolated colonies from which pure cultures may be made.

After the larvae have been dead for some time they become hard, brittle mummies; but, before any surface sporulation has occurred, isolations may be easily accomplished by dipping these in 95 per cent alcohol, quickly passing them through a flame, then cutting the larvae open with a sterile knife; the white mass of mycelium and spores can then be transferred in a relatively pure state to tubes of agar.

For comparative study *Beauveria Bassiana* and *B. globulifera* were grown in tubes of potato-dextrose agar and subjected to various temperatures. Similarly, these two organisms were grown in van Tieghem cells to determine the method and nature of spore germination. In all phases of this comparative study of the two organisms they were subjected to identical conditions.

GROWTH OF THE ORGANISMS ON ARTIFICIAL MEDIA

After observing the persistent, characteristic development of the stock cultures of these two organisms on potato-dextrose agar, it is obvious that *Beauveria Bassiana* always gives a mealy, chalky, pulverulent growth, while *B. globulifera* always produces an elevated, cottony, floccose growth (Fig. 1, D and E). It seemed advisable, therefore, to culture these fungi on various kinds of artificial media to see if these differences were lasting characteristics. The results are given in table 1.

From table 1 it is apparent that the fungus isolated from the corn borer always produces a characteristically flat, mealy, chalky, pulverulent growth on the various media used; while *Beauveria globulifera* produces a

TABLE 1.—*Summary of comparative development of Beauveria Bassiana and B. globulifera on artificial media. These two species were grown in the laboratory under identical conditions, and all transfers of each organism to these various substrata were made from the same stock culture*

Medium	Type of growth of B. Bassiana	Type of growth of B. globulifera
Potato-dextrose agar	Flat, chalky, mealy growth over whole slant	Loose, elevated, cottony growth over whole slant
[a]Bacto-prune agar	Flat, mealy, chalky growth. Slant ¾ covered	Loose, cottony, elevated to a less degree than above. Growth over whole slant
[a]Bacto-nutrient agar	Whole slant covered with flat, mealy, chalky growth. Agar purplish at base of slant	" " " "
[a]Proteose-peptone 2% agar added	Profuse, flat, mealy, pulverulent growth covering whole slant	Very profuse, elevated, loose, cottony growth over whole slant
[a]Bacto-peptone 2% agar added	" " " "	" " " "
[a]Lactose 2% agar added	Slight growth, slightly chalky. Lighter color than on proteose peptone	Slight growth, cottony, spheroid colony of hyphae
[a]Bacto-lactose 2% agar added	" " " "	Better growth than above, cottony, elevated
[a]Bacto-dextrose 2% agar added	" " " " and cottony	Slight growth, but elevated and cottony in patches
Corn meal	Little growth, lighter color than on proteose peptone	Little growth, slightly elevated, cottony colonies
[a]Bacto-bean-pod agar	Mealy, chalky growth. Base of slant well covered. Top covered in patches	Mealy, but somewhat elevated growth, of darker color than fungus from corn borer
[a]Bacto-malt agar	" " " "	Profuse, elevated, cottony growth, covering whole slant
Potato slab	Slightly cottony at first, soon becoming mealy, chalky, and flat	Profuse, elevated, cottony growth, in some cases turning potato slightly purple
Corn-meal mush	Flat, mealy, pulverulent growth	Profuse, elevated, cottony growth
Oatmeal	Slightly cottony at first, becoming flat, mealy, chalky	" " " "
10% gelatine	Flat, mealy growth. Gelatine turns brownish, amber color	Cottony, elevated growth

[a] Difco artificial media were used in this experiment.

characteristic elevated, cottony, loose, floccose growth, excluding the one culture on bean-pod agar, in which case the two organisms had a somewhat similar chalky appearance. Whether this character will change back again when the organisms are sown on an agar that normally gives an elevated, cottony growth has not yet been tried.

This consistently chalky, pulverulent growth on various media confirms the fact that this fungus, isolated from the corn borer imported from

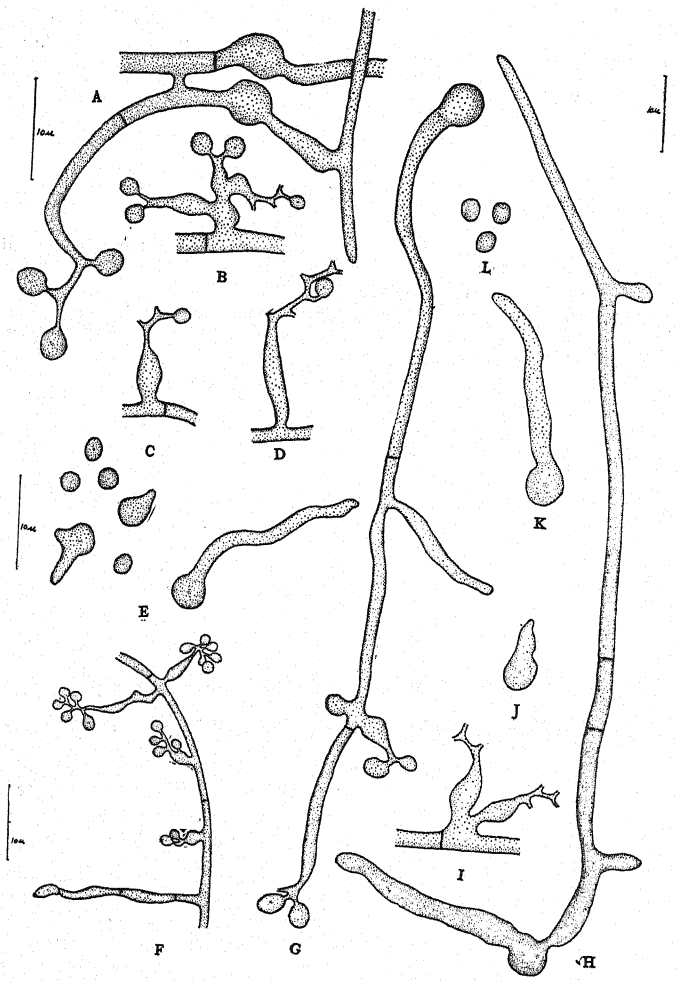


FIG. 2, A to G. *Beauveria Bassiana;* H to L. *B. globulifera.* A. Germinating spores with germ tubes anastomosing and one of them bearing conidia. ×1,350. B. Conidiophore branching into flask-shape phialides. ×1,250. C–D. Phialides: the one in C is flask-shape and that in D more slender, both having zigzag sterigmata. ×1,250. E. Four mature conidia, 3 showing pronounced swelling when germinating. ×1,250. F. Hypha bearing conidiophores and phialides; the spores are shown borne in clusters and singly. ×500. G. A germinating spore (after 32 hours) showing swelling of the conidia, and the septate, branching germ tube bearing conidia terminally and on a phialide. ×1,000. H. A germinating spore of *B. globulifera* (after 32 hours) showing swelling of the conidia, and the septate, branching germ tube; in this species, conidia are not yet formed. ×1,000. I. Conidiophore branching into flask-shape phialides showing zigzag sterigmata. ×1,250. J–K. Conidia showing pronounced swelling upon germination. ×1,250. L. Mature conidia at the time of shedding from the sterigmata. ×1,250.

Manchuria, is indeed *Beauveria Bassiana* (Bals.) Vuill. and is therefore distinct from *B. globulifera* (Speg.) Picard, common in this country. These findings agree very well with those of Petch and others who have done work on this group of imperfect fungi. This fungus, therefore, will be referred to as *B. Bassiana* throughout the remainder of this paper.

Another point of distinction between these two organisms, clearly brought out in the course of culturing them in the laboratory, was the difference in time required for spore formation. Observations on cultures of *Beauveria Bassiana* comprising data on 50 to 75 tubes of potato-dextrose agar grown under various conditions showed that abundant spore formation took place within a week, usually within 3 or 4 days; while in a like number of tubes of *B. globulifera* under identical conditions obvious spore formation did not take place until after the cultures had been growing 2 weeks to a month and then, only sparsely.

Spore germination: Studies of spore germination were made by placing conidia in drops of distilled water or 2 per cent peptone in van Tieghem cells following the method and percentage of germination. In 24 to 48 hours the spores swelled and put out one or more slender thin-wall germ tubes containing clear hyaline protoplasm. Frequently, they were found anastomosing (Fig. 2, A).

After 32 hours the germ tubes of *Beauveria globulifera* range from a few microns to as much as 200 μ in length, with the development of numerous branches 100 μ or more in length intertwining in a dense weft; while in *B. Bassiana* the germ tubes were much shorter, ranging from a few microns to about 80 μ, and the branches arising from these were very short. Consequently, the wefted appearance of the former was totally lacking in the latter. Thus, almost immediately in their early stages the mycelial development in van Tieghem cells substantiates what one finds when the two organisms are growing on agar, in that the tufted growth of *B. globulifera* and the more flattened growth of *B. Bassiana* are obvious.

At this stage of development conidial production is evident in *Beauveria Bassiana* (Fig. 2, G), while it is totally lacking in *B. globulifera* (Fig. 2, H). Later, when both organisms are producing conidia abundantly, they are borne in rather compact, globose heads, either on the main hyphal branches or on short laterals that are usually at right angles to the main axis, being much constricted at the base and terminating in an elongated, flask-shape conidiophore (phialide). Perhaps more commonly when these are at right angles to the main axis they occur in whorls and these also terminate in phialides and may bear branches in the same manner as those on the primary axis. Frequently this branching is repeated, forming compact heads whose make-up is difficult to follow (Fig. 3, A, B, C).

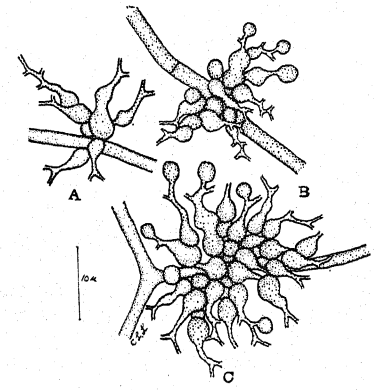


FIG. 3, A, B, and C. Hyphae bearing clusters of flask-shape phialides showing characteristic zigzag sterigmata. ×1,000.

Conidia are borne on short, slender, zigzag sterigmata borne at the apex of each phialide (Fig. 2, F). These, in turn, bear secondary sterigmata or short alternately arranged pedicels each of which bears a single conidium, and this may be repeated until a cyme of conidia is formed. These slender, zigzag, cymose sterigmata constitute the distinguishing character of the genus Beauveria (Fig. 2, C, D, and I).

In making observations on the percentage of spore germination, one was appalled at the small number of conidia that produced germ tubes, at all. The results of spore germination trials are given in table 2.

TABLE 2.—*Germination of spores of Beauveria Bassiana in distilled water and peptone*

Medium	Temperature	Per cent germination in center of droplet	Per cent germination edge of droplet
Sterile distilled water in 8 van Tieghem cells	Room	30, usually 10	90, unusual
Sterile distilled water in 6 Ward cells	Room	5	80 "
Sterile distilled water in 2 van Tieghem cells	10 C.	1 ±	1 ±
Sterile distilled water in 2 van Tieghem cells	27 C.	2	30
Sterile distilled water in 2 van Tieghem cells	33 C.	1 ±	1 ±
2 per cent peptone[a] in 2 van Tieghem cells	Room	50	90

[a] The writer has since secured much higher spore-germination percentages by placing the spores in peptone and dung decoctions.

Table 2 brings out the fact that the percentage of spore germination was somewhat higher when peptone was used as nutriment and that a greater number germinated around the edges of the droplet. When only a few spores were suspended in a droplet of the nutriment, germination seemed better; but too few observations were made to verify this fact, or it may be that the presence of large numbers of conidia in a droplet produces some antagonistic substances that have a tendency to lower the percentage of germination.

DIFFERENCES IN PATHOGENICITY OF BEAUVERIA BASSIANA, B. GLOBULIFERA, AND ISARIA FARINOSA

Because of manifest differences between *Beauveria Bassiana* and *B. globulifera* on artificial media and in the method of spore germination, it was thought that further contrasting characteristics might be obtained by trying inoculation experiments, using the corn-borer larvae as more or less of a differential host. Likewise, larvae were inoculated with *Isaria farinosa,* as this fungus is known to be parasitic on various insects (Table 3).

From table 3 it is apparent that there is a striking difference in the ability of these 3 organisms to infect the corn-borer larvae. Of the 50 larvae inoculated with *Beauveria Bassiana,* 30 died within 3 days when the larvae were thoroughly mixed and covered with spores by placing them in test tubes containing inoculum. By dusting, 6 out of 10 were killed within 5 days; and, when larvae were allowed to crawl over infected soil, 4 died within 6 days. In similar experiments in which 60 corn-borer larvae were inoculated with *B. globulifera* only 4 borers died from the disease. *Isaria farinosa* seemed to have no effect on the larvae. Controls in all cases showed no signs of infection. All these experiments were made at room temperature in the course of the summer, in the research laboratory of the Farlow Herbarium.

In another experiment 2 lots of 10 corn-borer larvae were dusted with spores of *Beauveria Bassiana* and *B. globulifera,* respectively; one lot was placed in the ice chest at 10–11° C. and the other placed in the 32–33° C. oven. There was no infection in either case, even though the 2 organisms grew on potato-dextrose agar at these temperatures. In all of these inoculation trials Petri dishes containing several layers of moist blotting paper were used as moist chambers, and string beans, thoroughly washed with sterile distilled water, were inserted in the chambers for food for the insects.

Arnaud (1) states that if silkworms, inoculated with *Beauveria Bassiana,* are kept in a moist chamber for 24 hours, then transferred to a dry container, the fungus will develop much better, and that if the infected

TABLE 3.—*Comparative pathogenicity of Beauveria Bassiana, B. globulifera, and Isaria farinosa on the corn borer in laboratory experiments*

	Date inoculated	Number inoculated	Method of inoculation	Date of infection noted	Number killed	Control	Remarks
Beauveria Bassiana	June 25	10	Dusting	June 30	6	No infection	4 others died but showed no disease
" "	July 6	10	Dusted surface of soil	July 12	4	"	
" "	" 12	10	Borers placed in test tube containing fungus	" 14	10	"	
" "	" 26	10	" " "	" 29	10	"	
" "	Aug. 1	10	" " "	Aug. 4	10	"	
" "	" 3	10	Dusting	.....	0	"	Placed in ice chest −11° C.
" "	" 3	10	Dusting	.....	0	"	Placed in 33° C. oven
B. globulifera	June 25	10	Dusting	.....	0	"	
" "	July 6	10	Dusted surface of soil	.....	0	"	
" "	" 12	10	Borers placed in test tube containing fungus	.....	0	"	
" "	" 26	20	" " "	.....	0	"	
" "	Aug. 1	10	" " "	Aug. 5	4	"	First time to be noted
" "	" 3	10	Dusting	.....	0	"	Placed in ice chest −11° C.
" "	" 3	10	Dusting	.....	0	"	Placed in 33° C. oven
Isaria farinosa	June 25	10	Dusting	.....	0	"	
" "	July 12	10	Borers placed in test tube containing fungus	.....	0	"	

worms are kept in a humid atmosphere continuously they will recover from the disease. This certainly does not hold true in the case of the corn borers, for, once they become infected, as first manifested by their becoming rather sluggish, failing to respond readily to external stimuli, and in some instances turning a "pinkish" color, none of these has ever been seen to recover.

Although only a few field experiments have been made, they indicate that *Beauveria Bassiana* is an organism possessing favorable potentialities for at least partial control of the corn borer in that the fungus readily infects and is destructive to the larvae yet grows well on artificial media and produces spores in vast numbers. Moreover, the corn-borer larvae, ensconced within the succulent tissues of the host during most of their period of feeding, live under relatively moist conditions, ideal for infection with fungous parasites. They are thus advantageously in contrast to certain other destructive insects against which fungous diseases have been used with little success on account of their feeding in the open under drier conditions. A summary of these field experiments and subsequent ones will shortly be compiled by Dr. K. Bartlett.

Spore characters: Since in the literature cited there is such a lack of agreement as to the size and shape of spores of *Beauveria Bassiana* and *B. globulifera,* measurements of 250 conidia were made by the writer, and the results were given in the following table.

TABLE 4.—*Summarized comparative measurements of conidia of Beauveria Bassiana and B. globulifera in water*

Length in μ	Classes *B. Bassiana*	*B. globulifera*	Diameter in μ	Classes *B. Bassiana*	*B. globulifera*
1.0–1.5	2	1	1.0–1.5	8	6
1.6–2.0	40	68	1.6–2.0	84	87
2.1–2.5	121	113	2.1–2.5	120	122
2.6–3.0	69	50	2.6–3.0	36	30
3.1–3.5	12	13	3.1–3.5	2	4
3.6–4.0	5	1	3.6–4.0	0	1
4.1–4.5	1	1			
4.6–5.0	0	1			
5.1–5.5	0	1			
5.6–6.0	0	1			

One notes at once that the larger number of spores fall in the class 2.1–2.5 μ for both length and diameter measurements. In general, the spores are globose but vary somewhat, as shown. Other writers give the following measurements:

Conidia globose:

Beauveria Bassiana (Bals.) Vuill.—

Conidia 2.5–2.8 μ (de Bary) (3)
" 2.0–2.5 μ (Delacr.) (4)
" 1.5–2.5 × 1.2–2.0 or globose
" 1.5 μ diam. (Petch) (5)

Beauveria globulifera (Speg.) Picard—

Conidia 2.0–2.5 × 1.5–2.0 μ (Speg.) (8)
" 1.74–2.5 μ (Pettit) (6)

From these data it is quite evident that spore size and shape are not a sound basis on which to distinguish *Beauveria Bassiana* from *B. globulifera* (Fig. 4).

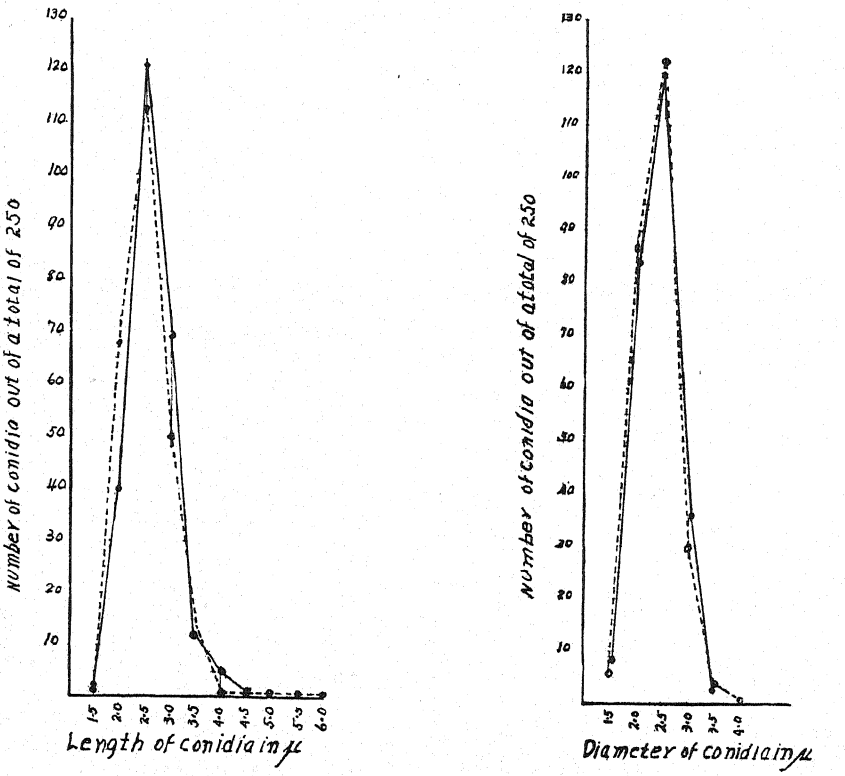


FIG. 4. Graphs showing comparative dimensions of the conidia of *Beauveria Bassiana* (———) and *B. globulifera* (– – – –) when grown and measured under identical conditions.

PRESENT KNOWN DISTRIBUTION OF THE FUNGUS

Beauveria Bassiana is known to be fairly wide-spread, as it occurs on insects in many localities, such as Europe, China, Japan, Ceylon, the Phil-

ippine Islands, and Manchuria. In fact, the fungus is so generally prevalent that it has been found impractical to ship corn-borer larvae from the district of Kanto, Manchuria, to the United States, where they are used to study the emergence of insect parasites. As far as the writer is aware, *B. Bassiana* has never been known to occur on the corn borer in the United States. The only record of this fungus in the United States is by Van Hook (9), who states that he found *Botrytis Bassiana* on a dead insect fast to a living leaf of cultivated strawberry; but, apparently, there is no mention that he cultured the fungus, nor is material available whereby further check on this determination can be made.

PERSISTENCE OF THE FUNGUS

The writer has not made extensive observations on the retention of vitality of the fungus, noting only that cultures kept in the laboratory 10 months are still capable of infecting the corn-borer larvae. This is in keeping with Arnaud (1), who states that by making frequent transfers cultures of *Beauveria Bassiana* were kept in the laboratory 2 years without altering their ability to infect the silkworm.

SUMMARY

Comparable cultural and inoculation experiments made with *Beauveria Bassiana* and *B. globulifera* have cast light of certain significance on the identity of these fungi (1) when grown on artificial media and (2) when used to inoculate corn-borer larvae.

Pure cultures of *B. Bassiana* are relatively easily secured, due to its fairly rapid growth and abundant spore formation.

On artificial media *B. Bassiana* is characterized by producing a flat, mealy, chalky, pulverulent growth, while *B. globulifera* forms an elevated, cottony, floccose growth.

In culture and on the corn borer *B. Bassiana* produces conidia much sooner and in greater numbers than *B. globulifera.*

On various agars and in van Tieghem cells *B. globulifera* has a greater mycelial development.

Beauveria Bassiana is a much more virulent pathogen on the corn borer than is *B. globulifera.*

For comparison with the two species of Beauveria, *Isaria farinosa* was tested under similar conditions but proved to be noninfectious.

Quantitative measurements of 250 conidia are given in tabular form, bringing out the close similarity in size and shape of the conidia of *B. Bassiana* and *B. globulifera.*

As far as is known, *B. Bassiana* has never been reported to occur on the corn borer in the United States but has been collected once in this country.

In cultures *B. Bassiana* does not seem to lose its virulence readily, at least not within the course of 10 months.

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BLIGHT OF CARROTS CAUSED BY SCLEROTIUM ROLFSII, WITH GEOGRAPHIC DISTRIBUTION AND HOST RANGE OF THE FUNGUS

GEORGE F. WEBER

A blight of carrots caused by *Sclerotium rolfsii* Sacc. was found near Gainesville, Fla., during the early summer of 1928. The disease appeared to be spreading in both directions in the rows from points of primary infection, killing the plant successively. This disease of carrots has not been previously described, so far as the writer has determined, although numerous hosts of the fungus have been reported.

DESCRIPTION OF THE DISEASE

The earliest symptoms of infected carrots are the general yellowness and lack of turgidity of the old leaves. As the disease develops these symptoms spread to the younger leaves, until, finally, all of them become involved. This development may require only 2 or 3 days under favorable conditions, yet the period is long enough to show distinctively the various stages in the advance of the disease. The older leaves wilt completely and become prostrate (Figs. 1 and 2), followed, successively, by the younger ones, until all of them lie on the soil surface. In this stage the leaves are loosely attached to the crown, and the root cannot be removed from the soil by pulling the leaves, while healthy roots are easily removed in this

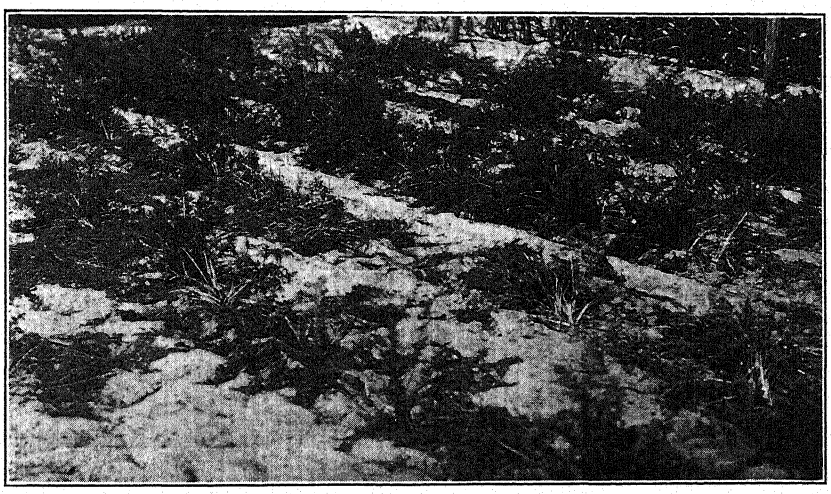


FIG. 1. Portion of a carrot field showing more than 50 per cent of the plants affected with *Sclerotium rolfsii* Sacc.



FIG. 2. Section of carrot row showing diseased plants compared with healthy ones at reader's right.

manner. They then lose their green or yellow color and become brown and crisp. The petioles easily become loosened from the crown of the plant and may be scattered by the wind, leaving no trace of the plant or the disease. There are no other symptoms above the soil surface by which the disease is characterized, except in fields where the plants have not been properly cultivated and portions of the crown are exposed. If such exposed roots are attacked, they often show white mycelial growth of the parasite. In contrast with this characteristic of the disease on carrots, the writer has observed that on a large number of other host plants that are vigorously attacked the causal fungus usually overgrows the stems of the plant from the soil line to several inches above. This condition has been reported by Higgins (15), McClintock (17), and Miller (19). Takahasi (36) also noted the mycelium of a similar fungus advance 6 inches up larkspur stems. Wolf (43), however, reported that the mycelium was entirely underground on peanuts, in Alabama.

The fungus invades the entire root of the carrot very quickly, causing a wet rot (Fig. 3). In some instances, where the decay had advanced through the periderm, cortex, phloem, and cambium, the central cylinder of pits and woody tissue would easily slip out when the leaves were pulled, leaving the outer cylinder of cortex and phloem in the soil. This outer portion of the carrot was held in the soil by the mycelium of the fungus, visible in the soil to a distance of an inch or more around the carrot. The distance the mycelial growth penetrated the soil was apparently determined by the amount of moisture supplied to the soil by the decaying carrot. The soil was usually wet from 1 to 2 inches around the carrots, which were from 1

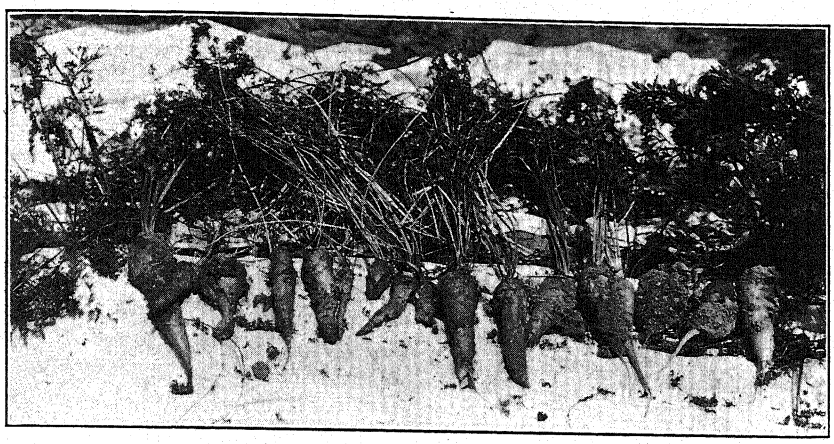


FIG. 3. The same plants shown in figure 2 removed from the ground and showing wet, soft decay.

to 2 inches in diameter. The distance around the smaller ones was relatively less. This condition appears to be of great importance in the spread of the disease in the row, as it enables the fungus to pass readily from one plant to another, even though the roots are not in direct contact. In advanced stages the decayed carrots dry out and shrink, leaving a cavity in the soil. The walls of the "sand-wells" thus found are held quite securely by the dried mycelium of the fungus. The sclerotia of the fungus are produced in large numbers on the deteriorating carrots in these cavities. Sclerotia were observed 6 inches below the soil surface, although not underground. Taubenhaus (37) recommended plowing 5 inches deep for the control of *Sclerotium rolfsii*. Under the above circumstances, however, such methods would probably place sclerotia on the surface rather than deep under the soil.

INOCULATION EXPERIMENTS

The fungus was obtained in pure culture by (a) planting mature sclerotia, which had been sterilized, on poured agar plates; (b) by transferring bits of the fluffy white mycelium that developed on infected roots (Fig. 4), kept for 24 hours in a moist chamber; and (c) by planting bits of invaded host tissue that had been surface sterilized. Fungus growth was evident on the plates after 12 hours. Within 4 days sclerotia began to develop. The mycelium and sclerotia (Fig. 5), in growth habits, color, size, and shape, were similar to descriptions given to *Sclerotium rolfsii* by Earle (9), Higgins (15), Rolfs (28, 29, 30), Saccardo (33), and Taubenhaus (37). Two weeks after the plantings were made in Petri dishes, the sclerotia produced therein were collected and used to inoculate carrots. Inocula-

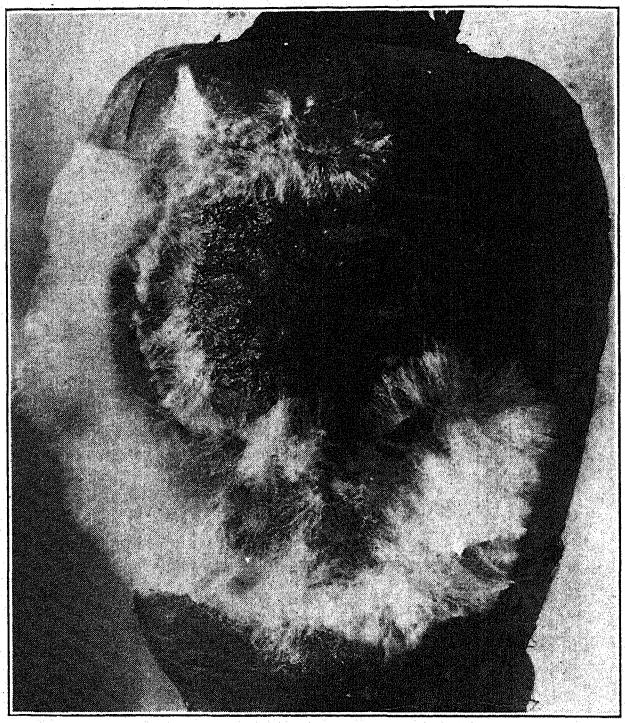


FIG. 4. A naturally infected carrot showing point of attack and the development of the fungus during a 24-hour period in a moist chamber.

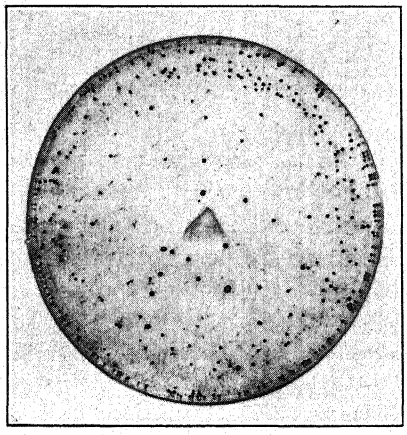


FIG. 5. *Sclerotium rolfsii* Sacc. in pure culture showing sclerotia.

tions were made in the field and in the laboratory, both with and without injury. A disease produced on inoculated plants was similar to that observed in the field.

TABLE 1.—*Results obtained by inoculating carrots with Sclerotium rolfsii from sources indicated*

Sources of inoculum	Parts inoculated	Number inoculated	Number inoculated	Checks	
				No.	Diseased
Sclerotia from field	Root	6	6	2	0
	Growing plant	12	10	2	0
Sclerotia from culture	Root	6	6	2	0
	Growing plant	12	11	2	0
Mycelium	Root	6	6	2	0
	Growing plant	12	6	2	0

In all cases of infection sclerotia were produced that were indistinguishable from those of the original culture. In moist chambers the inoculated carrots became overgrown with mycelium very rapidly. If they were left uncovered the mycelium developed more slowly but was much more dense. (Fig. 6).

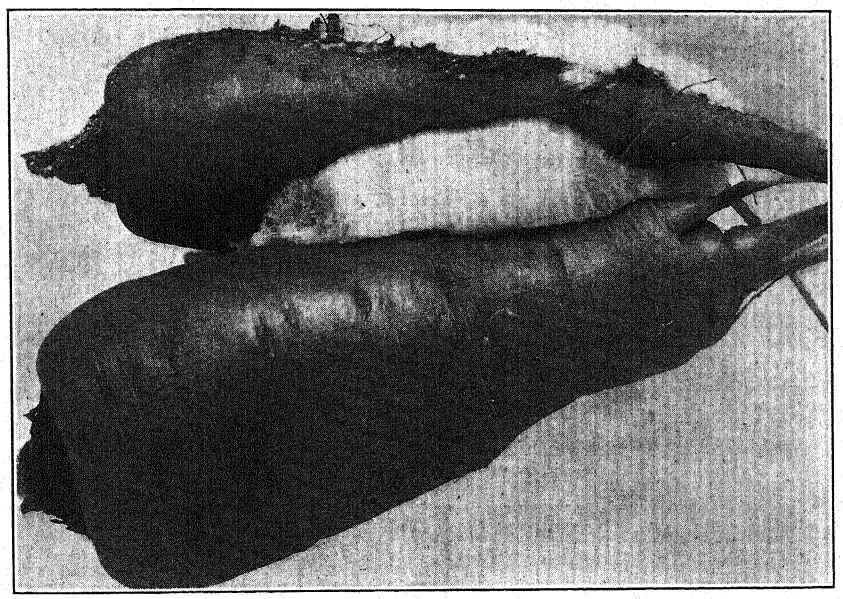


FIG. 6. A naturally infected carrot (above) producing the disease on the healthy carrot (below) by contact, in an open dish.

The disease has been collected in Florida on a large number of hosts, as listed hereafter, and in most instances pure cultures were made. A series of cross inoculations was made in which the fungus from various hosts was used to inoculate healthy plants, once reported as hosts. Table 2 shows the source of the inoculum used in the experiment, although the sclerotia employed as inoculum were developed in artificial culture. The results are given in the form of fractions in which the denominator represents the number of plants or plant parts inoculated and numerator the number of

TABLE 2.—*Results obtained by inoculating plants with Sclerotium rolfsii obtained from sources indicated*

Source of fungus	Plants inoculated												
	Bean	Beet	Carrot	Cotton	Cucumber	Eggplant	Lettuce	Pea	Pepper	Potato	Squash	Tomato	Watermelon
Bean	8[a]/10	4/6	5/10	4/10	7/10	4/6	6/6	4/4	4/6	4/4	6/10	10/10	4/6
Beet	4/6	8/10	6/6	4/4	7/8	6/10	4/4	6/6	5/6	2/4	4/6	6/6	5/6
Carrot	5/6	7/10	20/20	4/6	6/6	4/6	6/6	4/6	6/6	4/4	6/6	6/6	5/6
Cotton	3/6	8/10	10/10	10/20	5/6	5/6	6/6	5/6	6/6	3/4	5/6	5/6	6/6
Cucumber	3/6	4/6	8/10	4/4	6/10	4/4	6/6	4/4	4/5	3/4	4/4	4/5	4/4
Eggplant	4/6	4/4	6/6	4/6	4/10	10/10	6/6	6/6	—	6/6	5/5	6/6	2/6
Lettuce	6/6	3/4	3/4	4/5	1/4	2/5	10/10	4/6	—	3/4	3/7	4/4	5/5
Pea	4/6	4/6	8/10	4/4	4/4	4/6	10/10	10/15	—	2/2	3/4	7/7	4/6
Pepper	6/6	4/4	10/10	4/4	4/6	4/5	4/5	1/4	10/10	4/6	6/6	6/10	6/10
Potato	5/6	4/4	6/6	3/6	3/4	6/6	5/5	6/7	—	10/10	4/4	4/6	3/5
Squash	6/6	4/6	8/8	6/6	5/6	5/6	3/4	4/5	3/6	3/4	15/15	7/8	4/6
Tomato	6/6	3/5	10/10	4/4	4/6	6/6	6/6	3/3	6/8	4/4	6/10	15/15	5/10
Watermelon	4/6	1/4	6/10	4/4	3/3	5/5	7/8	4/6	10/10	3/6	4/4	4/4	10/10

[a] The denominators of the fractions designate the number of inoculations and the numerators the number of infections.

plants that became infected. The number of plants inoculated may include seedlings, grown plants, or the fruits or roots of plants. Check plants, not shown in the table, were used throughout the experiment. None of the plants was intentionally wounded in the inoculation work, but it is possible that microscopic wounds were present on such hosts as potato and carrot. In most cases sclerotia constituted the inoculum, although mycelium was used occasionally. The inoculated plants were grown in the greenhouse and in field plots.

These results show that the fungus is exceedingly cosmopolitan. The strain isolated from each of 13 plants was capable of infecting the other 12. There was no consistent difference among the strains, even though there was some variation in size, shape, and color of the sclerotia both in culture and on the various hosts, and, consequently, they are considered the same species.

GEOGRAPHIC DISTRIBUTION

A blight of various plants caused by *Sclerotium rolfsii* has been reported in certain foreign countries. In the Western Hemisphere the fungus and the disease it causes have been reported from the following: Porto Rico (16, p. 210), (35), Cuba (31), Hawaii (7), and southern portions of the United States (9, 15, 28, 37, 39). There is little doubt of its occurrence in the other subtropical and tropical lands of North and South America that are subject to excessive rainfall under high summer temperatures. Available reports of this fungus and its damage to living plants show that it occurs in Egypt (5), South Africa (20), India (8), (18), Australia (4), Japan (21), Sumatra (23), Philippines (2), Java (11), and Ceylon (3). In fact, the general group of lands thus reporting the disease are apparently those in the tropical and subtropical countries where high temperatures prevail during the rainy season. This belt, with slight variations both north and south, roughly follows the Equator, around the globe. The disease undoubtedly occurs and causes considerable damage in other countries located in this tropical or subtropical belt that are subject simultaneously to abundant rainfall and high temperatures. Definite reports from all such lands, however, are not available.

In the United States the disease is general in the Southern States from Texas to the Carolinas and north to the Ohio River, with occasional reports from scattered Central States. In Florida this disease is common and widespread on a large number of hosts covering the entire State, on its various types of soil. Blight is generally worse on the light, sandy types of soil during the rainy periods of summer, but this is not always true, since pepper and tomato fields have suffered considerable damage during January and February, on the heavy muck, marl, and loam soils.

HOST RANGE

Since the reports of the disease on different host plants are widely scattered in the literature it seems worth while to compile a list of the hosts reported from the United States and foreign countries.

The fungus has been collected on a large number of hosts. These hosts are here reported alphabetically, listing the Florida collections as well as others reported in the literature. The new Florida hosts, herewith reported for the first time, lack reference numbers.

HOSTS

Scientific name	Literature reference	Scientific name	Literature reference
Allium cepa L.	1	*Chrysanthemum* sp.	29
" *sativum* L.	1	*Cichorium endivia* L.	22
Alocasia cucullata Schott	3	*Cinchona* sp.	27
Amaranthus retroflexus L.	29	*Citrullus vulgaris* Schrad.	29
" *spinosus* L.	29	*Citrus aurantifolia* (Christm.) Sur.	26
Ambrosia artemisiifolia L.	29	" *decumana* L.	—
Amygdalus persica L.	1	" *grandis* Osbeck	—
Anacardium occidentale L.	—	" *limonia* Osbeck	26
Antirrhinum majus L.	—	" *paradisi* MacF.	26
Aquilegia sp.	40	" *sinensis* P.	26
Arachis hypogaea L.	29	*Cochranea anchusaefolia* (Poir) Gürke	—
Areca catechu L.	6	*Coffea* sp.	26
Arrhenatherum elatius (L.) Beauv.	39	*Coleus blumei* Benth.	22
Artocarpus communis Forst	35	*Colocasia esculenta* Schott	—
Asimina sp.	25	*Commelina* sp.	22
Avena sativa L.	40	*Coreopsis lanceolata* L.	—
Bambusa vulgaris Schrad.	35	*Cosmos* sp.	40
Bauhinia alba Buch-Ham	6	*Crataegus* sp.	—
Begonia sp.	22	*Crotalaria anagyroides* H. B. & K.	11
Beta vulgaris L.	29	" *juncea* L.	11
" *vulgaris cicla* Moq.	40	" *sericea* Retz	—
" *vulgaris macrorhiza* Stev.	1	" *spectabilis* Roth	—
Brassica juncea (L.) Cosson	25	" *usaramoensis* E. G. B.	—
" *oleracea* L.	29	*Cucumis melo* L.	—
" *oleracea acephala* (DC.) Kales	40	" *sativis* L.	—
" *oleracea botrytis* L.	—	*Cucurbita pepo condensa* Bailey	29
" *rapa* L.	—	*Cyamopsis tetragonoloba* Taub.	40
Bryophyllum pinnatum Kurz.	22	*Cynara scolymus* L.	5
Caladium bicolor Vent.	—	*Cynoglossum* sp.	—
Calendula officinalis L.	40	*Dahlia pinnata* Cav.	—
Callistephus chinensis Nees	—	*Daphne odora* Thunbg.	29
Campanula carpatica Jacq.	24	*Datura metel* L.	—
" *medium* L.	24	*Daucus carota* L.	—
" *nobilis* Lindl.	24	*Delphinium ajacis* L.	—
" *persicifolia* L.	24	" *grandiflorum* L.	—
Canavalia ensiformis DC.	14	*Desmodium molle* DC.	29
" *gladiata* DC.	23	" *tortuosum* DC.	29
Cannabis sativa L.	1	*Dianthus barbatus* L.	—
Capriola dactylon Kze.	—	" *caryophyllus* L.	—
Capsicum annuum L.	29		
Chenopodium sp.	22		

HOSTS

Scientific name	Literature reference
" *plumarius* L.	24
Dioscorea sativa L.	4
Dolichos multiflorus T. & G.	22
Dracocephalum argunense Tisch	24
Duranta plumieri Jacq.	—
Echinochloa frumentacea Link	22
Eleusine coracana Gaertn.	18
Erechtites hieracifolia (L.) Raf.	29
Erigeron canadensis L.	29
" *glabellus* Nutt.	24
Eupatorium ageratoides L.	24
Ficus carica L.	29
" *elastica* Roxb.	41
Forsythia sp.	40
Fragaria americana Brit.	—
" *virginiana* Duche	—
Gladiolus sp.	—
Glycine hispida Max	2
Gossypium hirsutum L.	—
Helianthus annuus L.	1
" *tuberosus* L.	—
Hibiscus cannabinus L.	13
" *esculentus* L.	25
Hippeastrum equestre Herb.	—
Holcus sorghum L.	1
Hordeum sativum Jess.	22
Hosta lancifolia Tratt.	40
Hydrangea paniculata Sieb.	29
Impatiens sultani Hook.	22
Indigofera endecaphylla Jacq.	34
" *hirsuta* Jacq.	34
Ipomoea batatas (L.) Lam.	29
" *purpurea* (L.) Roth	29
Iris sp.	40
" *xiphium* L.	—
Jasminum floridum Brenge	—
Lactuca sativa L.	—
Lagenaria leucantha Rusby	2
Lathyrus odoratus L.	—
Lespedeza simulata M. & B.	—
Lippia canescens Kunth	11
Lycopersicum esculentum Mill.	29
Malus sylvestris Mill.	4
Medicago sativa L.	1
Melilotus alba Desr.	22
Mimosa invisa Mart.	34
Musa sapientum L.	4
Myriophyllum sp.	22
Myrtus communis L.	—
Narcissus tazetta L.	—
" *tazetta orientalis* Hort.	—
Nicotiana rustica L.	20
" *tabacum* L.	42
Ornithogalum umbellatum L.	22
Oryza sativa L.	32
Oxalis sp.	22
Passiflora incarnata L.	—
Pentstemon murrayanus Hook.	24
" *pubescens* Soland.	24
Persea americana Mill.	—
Petroselinum hortense Hoffm.	22
Phaseolus vulgaris L.	29
Phlox subulata L.	24
Physalis angulata L.	—
" *pubescens* L.	—
Piper betle L.	38
" *nigrum* L.	—
Pisum sativum L.	29
Pittosporum tobira Ait.	—
Pteris longifolia L.	22
Raphanus sativus L.	3
Raphiolepis indica Lindl.	—
Reseda odorata L.	22
Rheum rhaponticum L.	29
Richardia scabra St. Hil.	—
Ricinus communis L.	22
Rosa rugosa Thunbg.	—
Rudbeckia laciniata L.	—
Saccharum officinarum L.	—
Salvia officinalis L.	22
Scabiosa atropurpurea L.	—
Scrophularia sp.	—
Sida rhombifolia L.	—
Soja max Piper	40
Solanum aculeatissimum Jacq.	—
" *gracile* Link	—
" *melongena* L.	29
" *mexicanum* D. & P.	—
" *munistrum* BiH.	—
" *nigrum* L.	—
" *tuberosum* L.	29
Stizolobium deeringianum Bort.	—
Tagetes sp.	—
Thymus vulgaris L.	22
Trifolium incarnatum L.	1
Triticum aestivum L.	10
Tropaeolum sp.	22
Vicia faba L.	35
Vigna catjang Walp.	1
" *hoei* Jacq.	34
" *oligosperma* Jacq.	—
" *sinensis* (L.) Endl.	3
Viola odorata L.	29
" *tricolor* L.	—
Xanthosoma sagittifolium (L.) Schott	12
Zantedeschia aethiopica Spreng.	—
Zea everta Sturt.	22
" *mays* L.	—
Zinnia elegans Jacq.	—

This list includes only a single report of each host. Where the host has been found in Florida, no other records are given. These hosts include succulent and woody, annual and perennial, and monocots and dicots. The fungus is more common on the succulent annuals, and it is not usually found on the woody perennials except under the most favorable temperature and moisture environment.

SUMMARY

1. A description of the disease caused by *Sclerotium rolfsii* Sacc. attacking carrots is given.
2. Isolations and inoculation experiments were conducted proving its pathogenicity.
3. Cross inoculations were conducted with 13 strains of the fungus, isolated from as many different plants, all of which proved susceptible to the various strains.
4. The strains of the fungus, although showing some variation in culture and on the hosts, did not warrant recognition.
5. The geographical distribution and host range of the fungus are given. One hundred and eighty-nine host plants are listed, 68 of which are reported here for the first time.

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GAINESVILLE, FLA.

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NEW APPLE-ROT FUNGI FROM WASHINGTON

GEORGE D. RUEHLE[2,3]

Beginning in the fall of 1926 and continuing through 1927, 1928, and 1929, a detailed study was made of the fungi causing decay of Washington apples in cold storage. Apples from representative districts obtained from commercially packed lots and placed in cold storage were given systematic examinations at intervals during their storage life and isolations of fungi made from the lesions which appeared. A total of 1,118 isolations have been studied. The following fungi have been isolated and proved by inoculation tests to be capable of causing decay either at cold-storage or higher temperatures:

PHYCOMYCETES

Mucor piriformis Fischer
Rhizopus nigricans Ehr.

ASCOMYCETES

Pleospora fructicola (Newton) Ruehle
Mycosphaerella tulasnei Jancz.

FUNGI IMPERFECTI

Phoma, No. 1
" No. 2
Coniothyrium, No. 1
" No. 2
Microdiplodia, sp. undet.
Gloeosporium perennans Z. & C.
Pestalozzia hartigii Tub.
Coryneum foliicolum Fckl.
Oospora, sp. undet.
Cephalosporium carpogenum, n. sp.
Penicillium expansum Lk.
" *puberulum* Banier
" *verrucosum* Biourge
" *olivino-viride* Biourge

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[3] The writer wishes to express his deep appreciation to Dr. F. D. Heald for able counsel and constructive criticism during the course of the work and for help in preparation of the manuscript.

Penicillium viridicatum Westling
" *martensii* Biourge, and 5 other unidentified species
Sporotrichum malorum Kidd & Beaum.
Sporotrichum carpogenum, n. sp.
Botrytis cinerea Pers.
Botrytis mali, n. sp.
Cladosporium malorum, n. sp.
" *herbarum* Lk. (See *Mycosphaerella tulasnei*)
Hormodendron cladosporioides (Fr.) Sacc.
Stemphylium congestum Newton
" " Newton, var. *minor* Ruehle
" (See *Pleospora fructicola*)
Alternaria tenuis Nees.
" *mali* Roberts
" No. 3
" No. 4
" No. 5
Fusarium No. 1
" No. 2
Ramularia magnusiana (Sacc.) Lind.
" No. 2
Epicoccum granulatum Penz.

BASIDIOMYCETES

Corticium centrifugum (Lev.) Bres.

It is not the purpose of this article to present the details of this study but to describe certain new species, to record some new data on previously described species, and to give brief attention to some not previously recorded as causing decay of apples.

NEW SPECIES

Cephalosporium carpogenum, n. sp. A species belonging undoubtedly in the genus Cephalosporium was found to be one of the less frequent weak parasites of apple fruit. It was obtained from small, shallow, dark brown, firm areas bordering worm holes or punctures. When reinoculated into ripe Jonathan apples held at about 20° C., small, shallow, firm spots were formed in 30 days. At the end of 2 months, a few of these spots had increased to 20 mm. in diameter on the surface, but most of them remained smaller, although the apples were very ripe. Pathogenicity was not determined at cold-storage temperatures.

The fungus develops slowly on culture media. On 2 per cent dextrose-potato agar, the colonies are white to faintly pink at first and become light gray in age (Fig. 1, A). The development of the pink color seems to be

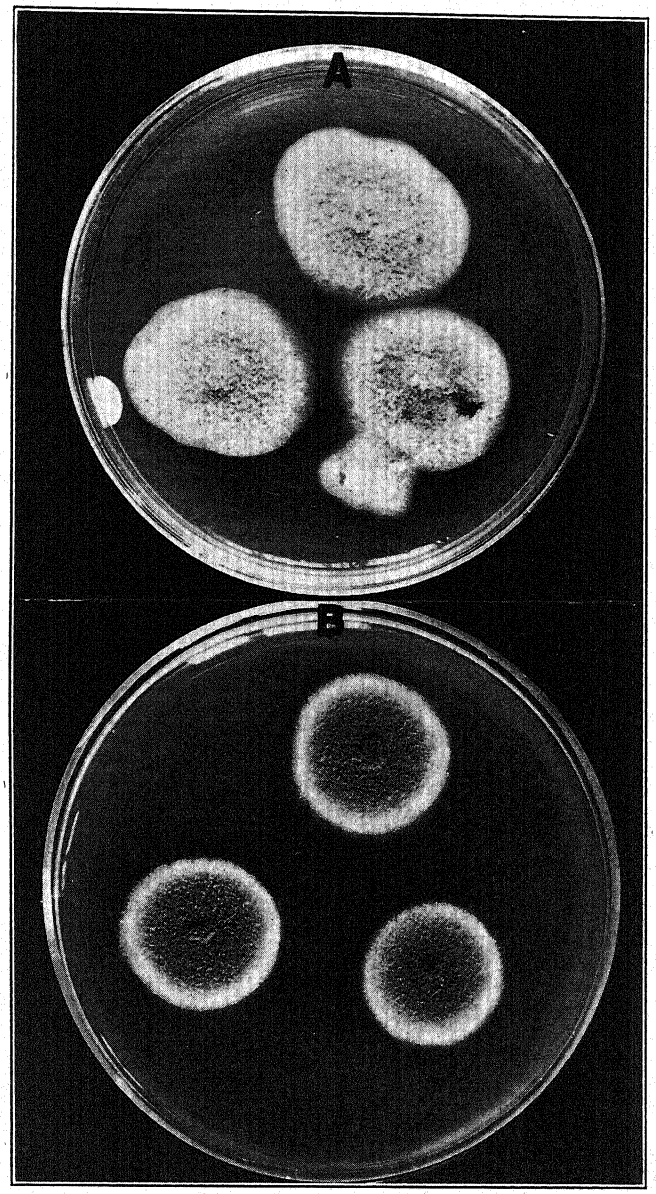


FIG. 1. A, *Cephalosporium carpogenum* on 2 per cent dextrose-potato agar; B, *Cladosporium malorum* on 2 per cent dextrose potato agar.

favored by growing the colonies in the light. The surface appears coarsely flocculate, bordered by a narrow zone of prostrate radiating hyphae. The hyphae are hyaline, sparsely branched, delicate, 1 to 2 μ in diameter, with septations very indistinct. In the aerial mycelium they are united into coarse strands composed of many parallel, adherent hyphae. The conidiophores are hyaline, simple, nonseptate, 1.5 to 2 μ wide at the base, and gradually attenuated toward the tip. They are 25 to 45 μ long and arise at nearly right angles from the hyphae. The conidia are hyaline, continuous, ellipsoidal to short cylindric, 4 to 8.5 μ long by 1.4 to 2.8 μ wide, produced successively on the apex of the conidiophores, but collecting there to form small, globose heads 8 to 15 μ in diameter, which break up readily in a water mount.

A single species of Cephalosporium has been previously reported from apple fruit in England by Kidd and Beaumont (6). This species, which they named *C. malorum* K. and Beaum., was always isolated associated with other fungi and there is nothing in their report to indicate that inoculation experiments were carried out with the fungus. In addition, the same investigators report two species of Hyalopus from apple fruit. Hyalopus is distinguished from Cephalosporium mainly by the tendency of the spore head of the former to remain intact, due to the greater amount of mucus formed. It has been suggested by Lindau (7) and Buchanan (1) that the two genera be combined, because this character is not considered sufficiently striking to warrant a separation.

A culture of *Cephalosporium malorum* was obtained from the Centraalbureau voor Schimmelcultures and was found on comparison to be distinct from the Washington fungus. The colonies develop much slower on culture media, and the spores are smaller (4 by 2 μ) in the English species. The Washington species also differs in spore characters from the two Hyalopus forms reported on the apple. It appears to be very similar to *C. charticola* Lindau, which has been described as occurring on moist paper, but the description of the latter is so inadequate that it is not possible to establish the identity of the two. It is, therefore, deemed advisable to consider the Washington fungus a new species, for which the name *Cephalosporium carpogenum* is proposed.

Sporotrichum carpogenum, n. sp. This form was isolated from a small, dark brown, firm, lesion on a Jonathan apple. It differs more or less distinctly from *S. malorum* (2), both in cultural characters and in size and shape of spores.

The colonies on 2 per cent dextrose-potato agar at about 20° C. spread very slowly, attaining a diameter of approximately 20 mm. in 10 days. In young colonies there is more or less fluffy overgrowth in the center, but later the aerial hyphae spread uniformly, forming a dense velvety mat of

a deep grayish olive (Ridgway XLVI) which becomes radiately furrowed in age. The margins are erose and without the border of white prostrate hyphae characteristic for *Sporotrichum malorum* (Fig. 2, C). The reverse side is dark olive buff to greenish olive in the deeper portions.

The hyphae are hyaline to light brown, 1 to 4 μ in diameter, with branches arising at short intervals. The hyphae are partly united into brown, coarse, strands of 3 to many parallel adherent hyphae. No loops or coils were observed in the aerial hyphae.

The conidiophores arise either singly as lateral outgrowths, usually at nearly right angles from the main hyphae, or more frequently in clusters of 2 to many arising at acute angles from short lateral branches from the main hyphae. Typically they are from 6 to 10 μ long and are swollen at the center and again at the tip to resemble a tenpin in shape, but occasionally the single conidiophores are much longer (up to 30 μ) and are scarcely distinguishable from the hypha from which they arise.

The conidia are subglobose to elliptical, hyaline, continuous, 2.8 to 6.4 by 1.8 to 3.5 μ. They are cut off successively from the apex of the conidiophore, and, in the aerial mycelium, accumulate about the tip of the conidiophore in a loose clump or "head," which breaks up readily in a water mount.

Because of the difference in rate of growth, character of colony, and size and shape of spores, this form is considered to be distinct from *Sporotrichum malorum*. It also differs in spore shape from *S. lyococcon* and *S. thümenii*, the two species listed by Oudemans (9) as occurring on apple fruit. It is, therefore, considered to be a new species, for which the name *Sporotrichum carpogenum* is proposed.

When inoculated into ripe Jonathan apples, this species was found to cause a dark brown, rather firm decay, at about 20° C. and at cold-storage

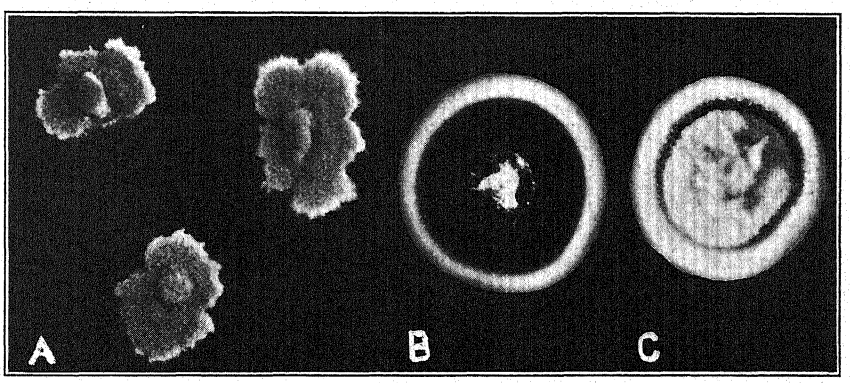


FIG. 2. A, Culture of *Sporotrichum carpogenum;* B, *S. malorum,* dark form; C, *S. malorum,* light form. All the same age on 2 per cent dextrose-potato agar.

temperatures. The rot lesions so produced are not markedly different from those produced by *Sporotrichum malorum* but were found to develop slightly slower at both temperatures. Spores and mycelium of the fungus were abundant in the host tissues, and reisolations yielded the original form in every case.

Botrytis mali, n. sp. This form is differentiated from *B. cinerea* by the production of very small sclerotia, by its shorter, more frequently branched conidiophores, and by its ovate to ellipsoidal conidia, produced on sterigmata which approximate half the length of the spores. By means of inoculation experiments on ripe apples, it was found to be capable of producing a rapid decay, which is of the same type as that produced by *B. cinerea,* but which develops slightly slower at all temperatures. The lenticel spotting noted for *B. cinerea* (3) apparently does not develop in lesions produced by the new form.

On 2 per cent dextrose-potato agar, the fungus grows somewhat slower than *Botrytis cinerea,* the colonies attaining a diameter of 75 mm. after 4 days' growth at a temperature of 25° C. The colonies are white at first, but soon become smoky gray from the production of numerous conidiophores and conidia (Fig. 3). Sclerotia begin to form in about a week and the colonies then gradually turn a light brown. The following is a technical description of the fungus as it develops on 2 per cent dextrose-potato agar.

Mycelium hyaline, septate, variable in diameter, the branches sometimes constricted slightly at the base; conidiophores erect, arising directly from the mycelium or sometimes from sclerotia, septate, simple or with numerous short side branches ending in globose swollen structures that bear conidia on small sterigmata; sterigmata attaining half the length of the spores or shorter; conidia continuous, hyaline, when single, but smoky gray in mass, standing close together to form dense heads; conidia ovate to ellipsoidal, usually finely apiculate at the base, 10–18 x 6.8–10.5 μ, average 11–14 x 7–9 μ; sclerotia white at first, becoming black at maturity, oval on top, flattened at the base, usually from 1 to 2 mm. in width but ranging from mere specks to 3 mm. (Fig. 3, B), germinating to produce conidiophores or to form a mycelium; attachment organs not formed in contact with Petri dish.

Cladosporium malorum, n. sp. Colonies on 2 per cent dextrose-potato agar, and Czapek's-solution agar, a Roman green (Ridgway's color standards), dense, with fluffy surfaces (Fig. 1, B), attaining a diameter of 60–65 mm. in 10 days when grown at 25° C. Hyphae are hyaline at first, becoming light olive; from 2 to 5 μ in diameter, branched, septate at short intervals, not constricted at the septa. The conidiophores are simple, septate, colored as the hyphae, and short. The conidia are light olive, oblong-cylindric with rounded ends, smooth-wall, mostly continuous, many 1-septate,

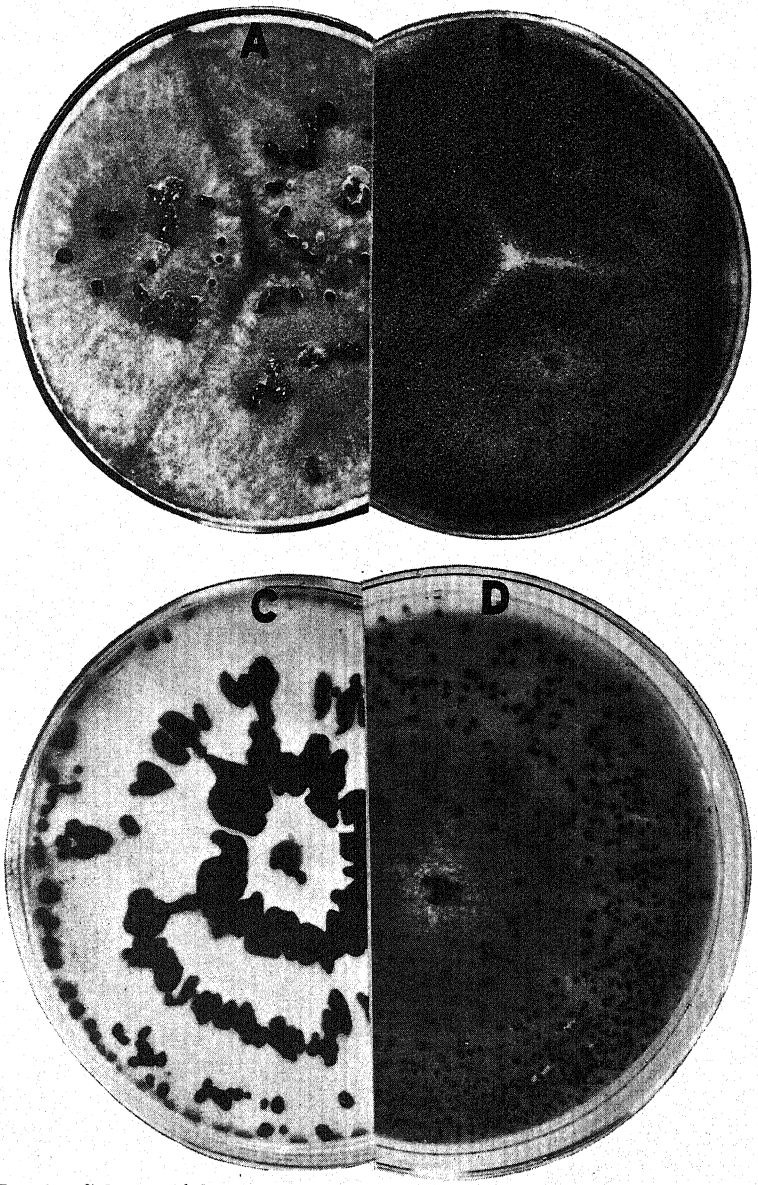


FIG. 3. Cultures of *Botrytis mali* and *B. cinerea* from both upper and lower surfaces. A, *B. cinerea*, upper surface with moderate sporulation; B, *B. mali*, upper surface with heavy sporulation; C, *B. cinerea*, lower surface showing large sclerotia; D, *B. mali*, lower surface showing numerous small sclerotia.

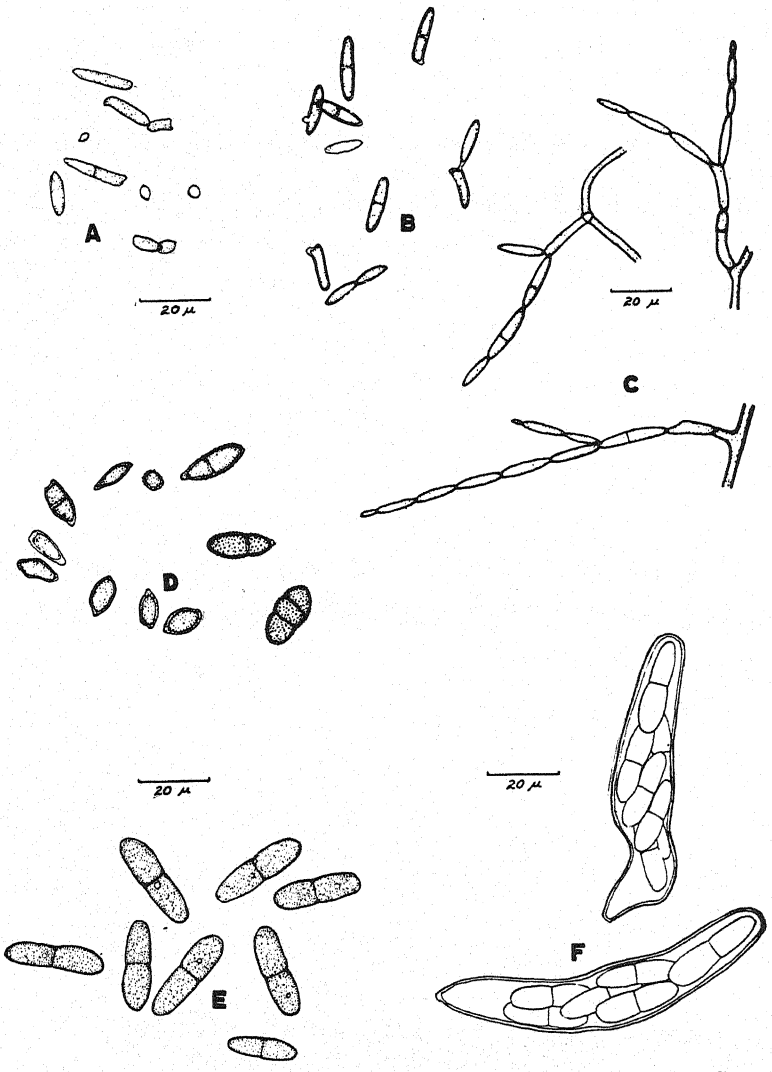


FIG. 4. Drawings of Cladosporium-like forms from apple rots. A, Conidia of *Hormodendron cladosporioides;* B, mature conidia of *C. malorum;* C, young conidial chains of the same species; D, conidia of *Mycosphaerella tulasnei;* E, isolated ascospores of *M. tulasnei;* F, two asci of this species containing ascospores.

produced in long branched chains. Each conidium arises as a bud on that immediately behind, so that the youngest conidium is at the distal end of the chain. The spores are 10–21 x 3–5 μ, the average being 14.7 by 3.6 μ (on the basis of 100 spores), (Fig. 4, B, C).

Inoculation experiments showed that the fungus is capable of producing decay of ripe apples. At 20–25° C., dark brown lesions 20–30 mm. in diameter were formed at the end of 14 days. The rotted areas are fairly firm, and the affected tissue is light brown, rather dry and spongy. The original form was recovered from tissue plantings made from the advancing edge of such lesions. At 0° C., the fungus develops very feebly on the apple, producing small spots at the points of inoculation, which do not spread to cause decay. Although inoculated apples were incubated for 5 months at this temperature, these spot lesions did not advance beyond 10 mm. in diameter. The fungus is able to grow on culture media at this temperature, however, producing colonies that attain 35 mm. in diameter at the end of 2 months. Such colonies are loosely fluffy and produce very few spores.

MYCOSPHAERELLA TULASNEI AND CLADOSPORIUM SPECIES

In the present study fungi of the Cladosporium-Hormodendron type were repeatedly isolated from small, brown to black, firm, shallow lesions developing in apples in cold storage. The lesions did not exceed 20 mm. in diameter and occurred invariably at breaks or injuries to the skin of the fruit, such as bruises, stem punctures, insect injuries, or scalded areas. The morphology of the various isolations was studied from colonies developed from single spores. Eleven strains or species were recognized, possessing fairly constant characters when grown more than a year on culture media. Of these only three were found capable of causing decay when inoculated into apples, as follows:

1. *Cladosporium malorum* described above as a new species.
2. *Cladosporium herbarum* Lk. Colonies very dense with scant development of aerial hyphae, conidia variable in size and shape, 0–3 septate, with roughened walls in branched chains (Fig. 4, D).
3. *Hormodendron cladosporioides.* Similar to No. 2, but conidia smaller; 0–2 septate, smooth (Fig. 4, A).

Although types 2 and 3 have been considered by various writers as stages of the same fungus, it should be emphasized that with frequent transfers and growth at varying temperatures for a period of 3 years, they retained their specific characters. No. 2 developed a perithecial stage, identified as

Mycosphaerella tulasnei Janc., but No. 3 could never be induced to form perithecia.

In 1894 Janczewski (5) described one form of *Cladosporium herbarum* as belonging to the life cycle of *Mycosphaerella tulasnei*, but no confirmation of an ascigerous stage of *C. herbarum*, since that first report, has been found in any of the literature studied. This early discovery of Janczewski appears to have been either ignored or received with some doubt by modern writers. The definite confirmation of this report should be of interest.

Mature perithecia were produced in small numbers on culture media such as corn-meal agar or potato-dextrose agar, when held at low temperatures for long periods, but more abundantly on sterilized wheat leaves inoculated with spores of the Cladosporium stage and incubated at 8–10° C. for a period of 6 months. These perithecia agreed, in general, with those originally described by Janczewski.

Single ascospores were isolated and grown in culture, with the resulting colonies similar in all respects to the original colonies grown from single conidia. There seems little doubt that this form isolated from the apple is the same as the species described by Janczewski, although he reported somewhat larger perithecia and larger asci. At the end of 6 months the perithecia on sterilized wheat leaves were black, with thick wall, typically broadly flask-shape with a short neck, and partially embedded in the leaf tissue, 150–250 μ high by 100–150 wide. The asci are cylindrical, slightly tapering at the ends, 80–120 x 15–20 μ; ascospores hyaline, bicellular, 18–28 x 6–8.5 μ (Fig. 4, E, F).

ADDITIONAL NOTES

In 1928 Newton (8) described a new species of Pleospora from decaying apples in the Pacific Northwest and named it *Pleospora mali* Newton. The conidial stage was classified as a Stemphylium. *Pleospora mali* Newton should not be confused with *P. mali* Hesler, an entirely different species, recently described as the perfect stage of *Hendersonia mali* Thüm (4). Since the published description of *P. mali* Hesler antedates a few months the account by Newton, the specific name given by Newton to the apple-rotting Pleospora must be considered invalid. *Pleospora fructicola* (Newton) Ruehle is proposed for the species described by Newton.

A fungus of the Microdiplodia type has been obtained several times from small, dark brown, firm lesions (20 mm. or less) occurring at punctures. Cultures on potato-dextrose agar fruit readily, especially at temperatures of 10–15° C. The colonies at first are white, but soon become olive brown, with white advancing border. The aerial mycelium, which is white at first, becomes dark brown with age and is partly united into small compact

strands consisting usually of 4–6 hyphae. The pycnidia are scattered, or sometimes in groups of 2 or 3, subglobose, nonostiolate, dark brown, 100–230 μ in diameter with thin, easily ruptured walls 2–3 cells thick. The pycnospores are light to dark brown, usually 1-septate (occasionally 2–3) oval to oblong with rounded ends, smooth wall and 9.5–15.5 x 6.9–9.5 μ. Inoculation of ripe Jonathan apples at laboratory temperatures gave brown, firm, shallow lesions which reached a diameter of 25 mm. or less in 60 days. No pycnids were formed on the fruit lesions. No records of the previous occurrence of a Microdiplodia causing apple decay have been found.

A species of Epicoccum, agreeing closely with *E. granulatum* Penz., was obtained from a dark rot on a Rome Beauty apple. When inoculated into ripe Jonathan apples it produced a firm, rather dry, reddish brown rot in which cavities were sometimes formed, both in cold storage and at 20° C. In cold storage the lesions were 10–35 mm. in diameter after 5 months, while at the higher temperature they were 20–25 mm. in diameter after 2 months. The affected tissue was sometimes reddish brown and dry, but in larger lesions it was soft, moist and bright red. No species of Epicoccum have been previously reported as capable of causing decay in apples.

Several fungi have been isolated from apples which have not been assigned to definite species. These appear to be distinct from species of these genera previously recorded as affecting apples, but it has not seemed advisable to give them specific rank until they can be given a more detailed study. The isolations falling in this class are:

Phoma Nos. 1 and 2.
Coniothyrium Nos. 1 and 2.
Oospora sp.
Alternaria Nos. 3, 4, and 5.
Fusarium Nos. 1 and 2.
Ramularia No. 2.

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A COMPARISON OF PSEUDOMONAS PRUNICOLA WITH A CANKER-PRODUCING BACTERIUM OF STONE-FRUIT TREES IN CALIFORNIA

E. E. WILSON[1]

INTRODUCTION

In 1928 Wormald (8) described a wilt disease of the green shoots of plums in England and later (9) presented proof that this disease is caused by a bacterium, to which he gave the name *Pseudomonas prunicola,* n. sp. The organism differed in major characteristics from *Bacillus spongiosus* Ader. and Ruh. and from *Bacterium* (*Pseudomonas*) *pruni* E. F. Smith. It also differed in certain details from *Pseudomonas cerasi* Grif., as described by Griffin (6) and Barss (2).

The present paper describes the results of a comparative study of *Pseudomonas prunicola* Wormald, with an organism found commonly associated with a gummosis of stone-fruit trees in California. The latter organism possesses distinct pathogenic abilities but does not possess the chromogenic ability attributed to *Ps. cerasi.* Due to this difference and the fact that a second type of organism has been found that does possess the chromogenic ability, the writer prefers, for the present at least, to designate his organism as 357. These points will be discussed more in detail in the body of the paper.

Dr. Wormald kindly furnished the writer with a culture of *Pseudomonas prunicola.* The culture of 357 was obtained from blighted apricots buds during the winter of 1929–30.

MORPHOLOGICAL AND STAINING STUDIES

Both *Pseudomonas cerasi* (6) and *Ps. prunicola* (9) are described as rods motile by 1 to 3 polar flagella and commonly occurring in pairs. The former is reported to be from 1.5 to 2.4 μ long by 0.5 to 0.84 μ wide; the latter, 0.9 to 2.5 μ long by 0.3 to 0.5 μ wide.

In the present study 357 and *Pseudomonas prunicola* were grown comparatively on beef-extract agar. Smears taken at 48 and 120 hours were stained with safranin. Table 1 presents the results of measurements by two persons. Only those cells were measured that showed neither a constriction nor a transverse separation. In this way, errors arising from measurements of cells in the process of division or of connected cells were minimized.

The data of table 1 show no consistent difference between the size of the two organisms. The differences obtained by the two observers of measure-

[1] The writer wishes to acknowledge his indebtedness to Professor R. E. Smith for advice and criticism.

ments of cells in the same smears were probably incidental to their abilities to estimate fractions of a space on the ocular micrometer.

Wormald (9) states that *Pseudomonas prunicola* may at times become linked together in long chains, a characteristic which has also been noted for 357. The phenomenon occurred in beef-extract agar cultures of 357, which had been growing at room temperature for 120 hours. When such chains were stained with methylene blue, it was seen that the organisms were held together by a common hyaline sheath.

Wormald (9) found that *Pseudomonas prunicola* was slightly Gram positive if he used either the method of Eyre or those methods outlined in the S. A. B. Manual. In the present work, smears of 357 and *Ps. prunicola* grown on beef-extract agar were taken at intervals and stained by the aniline-gentian-violet method outlined in the S. A. B. Manual. Although both organisms retained some of the gentian violet, the method was deemed unsatisfactory, inasmuch as the stain was precipitated as particles over and around the cells. When the procedure was modified by washing the smears in water between steps, this precipitation did not occur, nor did the cells retain the stain.

TABLE 1.—*Measurements by two persons of 357 and Pseudomonas prunicola grown for different lengths of time on beef-extract agar*

Organism and age in culture	Observer No. 1			Observer No. 2		
	Extremes of length	Extremes of width	Average length and width	Extremes of length	Extremes of width	Average length and width
	Microns	Microns	Microns	Microns	Microns	Microns
357:						
48 hours	1.1–2.5	0.5–0.7	1.8 × 0.7	1.1–2.8	0.5–0.7	1.6 × 0.7
120 hours	0.9–2.3	0.5–0.7	1.7 × 0.5	0.8–2.8	0.5–0.7	2.2 × 0.7
Ps. prunicola:						
48 hours	1.1–2.8	0.5–0.7	2.1 × 0.7	1.1–3.2	0.5–0.7	1.9 × 0.5
120 hours	1.1–2.8	0.5–0.7	1.8 × 0.5	1.3–2.8	0.5–0.7	1.8 × 0.6

CULTURAL STUDIES

Pigment production.—Wormald (9) did not consider *Pseudomonas prunicola* to be identical with *Ps. cerasi* inasmuch as the latter was reported to produce a green pigment, while the former produced a lemon-yellow pigment, which diffused through the medium, leaving the mass of organisms uncolored.

The various workers on the gummosis of stone fruit are not definite as to the chromogenesis of the organisms they found causing the disease. Griffin (6) (see also Barss, 2) states that the greening of a number of

media was a constant characteristic of *Pseudomonas cerasi*. Barss (2) did not report results of his cultural studies but was evidently in accord with Griffin on this point. Barrett (1) does not give the cultural characteristics of the organism he found to be the cause of apricot and plum gummosis in California. Goldsworthy (5), working in California, reported a fluorescent and a nonfluorescent type capable of producing gummosis. The fluorescent characters was not constant. While the two types were indistinguishable by cultural tests he was able to show that they produced specific agglutinins in experimental rabbits. Smith and Fawcett (7) state that their isolation of *Ps. cerasi* differed in its utilization of lactose and maltose from that reported by Griffin but do not mention pigment production.

The organism (357), which has been found in the present study to be most common in gummosis cankers, produced a yellow rather than a green discoloration in beef-extract media. On potato-glucose agar only a slight yellow tinge appeared after the organism had been growing for a number of days. This characteristic on potato glucose distinguished 357 from a second type (506), which has been found less frequently. On this medium, and to a lesser degree on beef-extract media, the second type produced a brilliant yellowish green pigment (approaching an apple green by reflected light; Ridgway[2]) that possessed fluorescent qualities. This type has produced gumming cankers upon being inoculated into plum and apricot trees and has been recovered from such cankers several months later.

It is beyond the scope of this paper to deal with the importance of the two types in the general problem of gummosis. A more exhaustive study is necessary before this phase can be properly evaluated. Their existence has been mentioned in order to show why the writer is unwilling to designate the more common one (357) as *Pseudomonas cerasi*. As shown later, 357 also differed from *Ps. cerasi* in its utilization of lactose and maltose.

In a series of tests 357 and *Pseudomonas prunicola* were similar in type of growth and discoloration of beef-extract media. After 7 to 10 days the medium was changed to a lemon-chrome or lemon-yellow (Ridgway[3]).

In a medium composed of 0.5 gm. ammonium dihydrogen phosphate, 0.2 gm. potassium chloride, 0.01 gm. calcium chloride, and 10 gms. succinic acid per liter and adjusted to pH 7.0 with sodium hydroxide, both organisms produced a characteristic greenish yellow color. While the color did not possess marked fluorescent qualities by daylight examination, it would probably fall into this category.

[2] Ridgway, Robert. Color standards and color nomenclature. Washington, D. C. 1912.

[3] *Op. cit.*

Pseudomonas prunicola differed from 357 in these tests only in becoming somewhat flocculent by the 6th day, while 357 remained evenly suspended.

Utilization of nitrates.—Following the procedure outlined in the S. A. B. Manual, a study was made of the growth of 357 and *Pseudomonas prunicola* on media containing nitrates. A preliminary test had failed to show the reduction of nitrates to nitrites. The next step was to grow the organisms on four different media as follows: (a) potassium nitrate-beef-extract broth; (b) beef-extract broth without the addition of potassium nitrate; (c) a synthetic medium containing potassium nitrate, dipotassium phosphate, calcium chloride, and glucose; and (d) a beef-extract broth containing 2 parts per million of potassium nitrite. Using the reagents recommended in the Manual, "a" and "b" were tested for ammonia, "c" was tested for both ammonia and nitrites, and "d" was tested for nitrites.

The tests gave no indication that either organism produced nitrites or ammonia. Upon applying the test for ammonia to a and b, a yellowish color appeared in the bottom of the tubes. This slowly changed to a green, which lasted for 20 to 30 minutes and then disappeared, but no blue color appeared at any time. There was evidence that both organisms were capable of utilizing nitrite, as the test for this substance in d became weaker and weaker until by 96 hours no test could be obtained.

Utilization of various carbon compounds.—Pseudomonas cerasi was originally described (Barss, 2) as producing acid on lactose and maltose. This was expressed in terms of the titratable acidity (Fuller Scale). Smith and Fawcett (7), basing their tests on the change in pH, have reported that their isolation of *Ps. cerasi* produced alkali on these sugars.

Wormald (9) found that *Pseudomonas prunicola* was able to grow in "nutrient broth" to which no carbohydrate had been added. The medium under these circumstances became more alkaline. If either glycerin or lactose was added to the above medium the bacteria caused the pH to be shifted towards the alkaline side. If, however, either glucose or sucrose was used the bacteria shifted the pH towards the acid side.

The experience of the writer has been that, if the bacteria produce small amounts of acid from a given carbon source, its presence may be masked by the alkali produced from their action on the peptone, if peptone is used in the medium. Thus, it was found that in beef-extract broth with glycerin as a carbon source, 357 often produced alkali, while in a medium composed of inorganic salts and glycerin, the organism produced acid. Studies of the utilization of carbon compounds were therefore made on both types of media.

Table 2 presents data obtained by growing the two organisms on beef-extract agar with various sugars as carbon sources. The medium was ad-

justed to a reaction of pH 6.5, with brom thymol blue as an indicator. With the exception of the glycerin medium, the two organisms produced very similar changes of pH.

TABLE 2.—*Comparison of change in pH produced by 357 and Pseudomonas prunicola in beef-extract agar containing different carbon sources*

Carbon source	Organism	Change[a] from control after stated time				
		2 days	3 days	5 days	8 days	15 days
Galactose	*Ps. prunicola*	0	+++	+++	+++	+
	357	++	+++	+++	+++	+
Glucose	*Ps. prunicola*	++	+++	+++	+++	−
	357	++	+++	+++	+++	−
Glycerin	*Ps. prunicola*	−	−	0	0	+
	357	0	+	++	++	++
Lactose	*Ps. prunicola*	−	−	−	−−	−−−
	357	−	−	−	−−	−−
Sucrose	*Ps. prunicola*	+	+	++	++	−−
	357	+	0	++	++	−

[a] 0 = No change from pH 6.5; (+) = change towards acid side; (−) = change towards alkaline side.

The general appearance of the two organisms in the agar streaks was strikingly similar. One rather common characteristic was the occurrence of minute pits in the surface of the growth, which resulted in a rather dull appearance (matte-effect). The condition did not always persist but gave place to a smoother and more glistening surface. The growth was rather flat on all of these media; the margins were generally slightly lobed and the lobes, in turn, were irregularly toothed. In the case of the dextrose and glycerin media, the margins tended to be more entire.

In the inorganic medium mentioned under "Pigment production," both organisms shifted the pH towards the alkaline side when the carbon source was either lactose, raffinose, trehalose, or succinic acid. When either glucose or sucrose was used there was an increase in hydrogen-ion concentration. Neither organism appeared to be able to utilize rhamnose to any great extent, as no growth had occurred in this medium after 72 hours. Figure 1 shows graphically the results obtained in the glucose medium. While the curves do not parallel each other at all points, there is a general agreement in their conformation, in that both organisms produced a rather slow in-

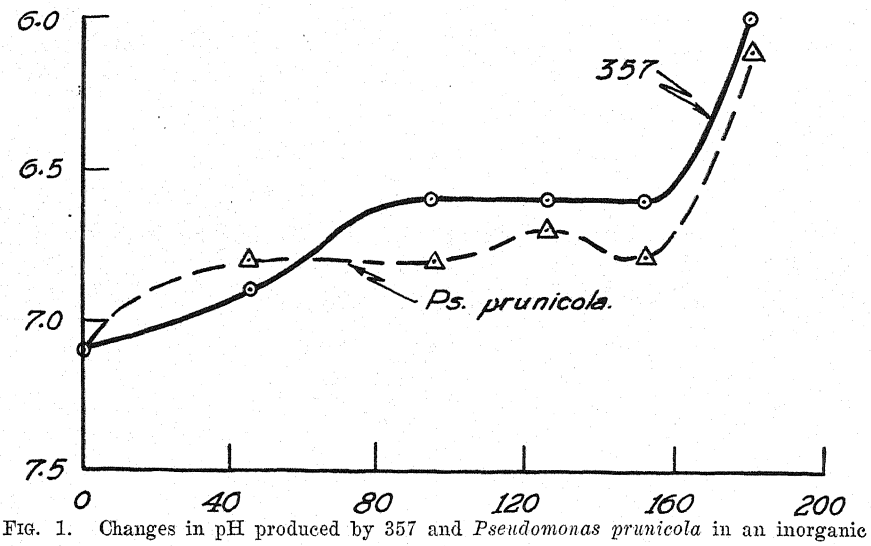


FIG. 1. Changes in pH produced by 357 and *Pseudomonas prunicola* in an inorganic liquid medium containing glucose as a carbon source. Hydrogen ion concentration plotted against time in hours.

crease in hydrogen-ion concentration during the first 148 hours, after which there was a rather sudden increase.

PATHOGENICITY

Pseudomonas cerasi is capable of attacking under natural conditions the leaves, the buds, and limbs of stone fruits (Barrett, 1; Barss, 2, 3, 4). It has not been reported to attack the green shoots in nature but has been found by Smith and Fawcett (7) to produce lesions on the green shoots of a rather wide variety of hosts when inoculated artificially. Even though natural infection of green shoots was possible, the low humidity conditions of the Pacific Coast during the growing season would probably militate against an abundance of this type of injury.

Wormald (9) stated that *Pseudomonas prunicola* produced gumming cankers when inoculated into the limbs of plum but that these cankers never became large enough to girdle the limbs. He (9) further observed that *Pseudomonas prunicola* had never been found associated with naturally occurring cankers on "woody stems" of the plum but mentions a second bacterium that did produce extensive natural cankers. The description of the second bacterium was reserved for a later publication.

Since *Pseudomonas prunicola* and 357 agreed in most of the culture tests used, the two organisms were inoculated into the limbs of plum and cherry trees by means of a hypodermic needle. The small wounds thus made were covered with either vaseline or oiled paper.

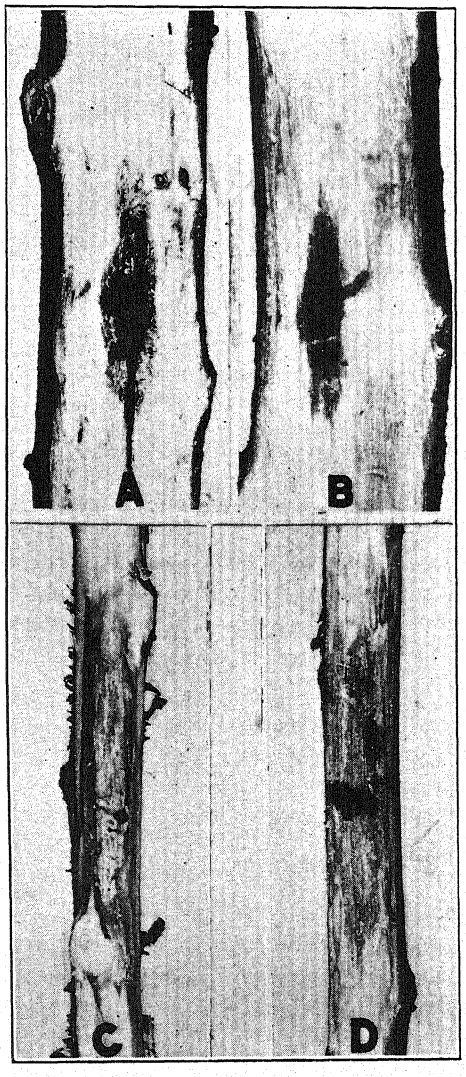


FIG. 2. Comparison of cankers produced by (A) 357 and (B) *Pseudomonas prunicola* on Duarte plum branches and by (C) 357 and (D) *Ps. prunicola* on President plum branches. $\times \frac{2}{3}$.

In a series of 36 inoculations into Duarte plum on January 14, 1931, there was never much evidence of gumming from the points of inoculation, but by February 18 depressions were noticeable around the wounds. Upon cutting into the bark definite cankers were found that were from 1 to 3 inches long and from ½ to 1 inch wide. Both organisms produced cankers similar in all respects (Fig. 2, A, B). It was characteristic for gum to be exuded both from the edge and in advance of the necrotic areas of these cankers when the bark was cut.

On January 15, 1931, 20 inoculations were made into young German Mazzard cherry trees, and 36 inoculations were made into young trees of Grand Duke, President, and Wickson plums. Neither 357 nor *Pseudomonas prunicola* produced cankers over 1 inch long on the cherries. On the plums, however, both organisms had produced by March 3 cankers from 3 to 6 inches long (Fig. 2, C, D). These cankers were similar in all respects. The most rapid progress had been made along the cambium. The edges of the cankers were generally watery or gum-impregnated, while the centers were dark brown. Gum-soaked streaks occurred through the phloem region and would often terminate in gum pockets.

A series of 15 inoculations was made into limbs of Bing cherry on January 15, 1931. By January 25 abundant gum had been exuded from the point of inoculation. When the final observations were made on March 5 it was found that both organisms had produced cankers from 2 to 3 inches long and from ¾ to 1 inch wide. Gum pockets were present at times 2 or more inches from the point of inoculation.

Organisms that agreed in all respects with those used for inoculations were recovered from these cankers. It was comparatively easy to obtain a pure culture by removing bits of bark from the edges of the cankers and dropping them into broth tubes. The same was true if such bits of bark were allowed to stand in sterile water for an hour or two, after which platings were made from the water.

SUMMARY AND DISCUSSION

A comparative study has revealed that *Pseudomonas prunicola* resembles very closely an organism, designated in this paper as 357, which produces a gummosis disease of plum and apricot trees in California.

The latter organism differs in certain cultural details from *Ps. cerasi*, the reported cause of gummosis of cherry trees in Oregon.

A second type of organism (506) is noted in California, which resembles *Ps. cerasi* more closely in chromogenesis. It is also capable of producing a gummosis type of canker on plum and apricot. More detailed work is necessary to determine the relationship of the two types to each other and to *Ps. cerasi*.

The work of Goldsworthy (5) is cited as indicating that two very similar organisms may cause gummosis of stone-fruit trees. Since this worker failed to report the cardinal features of his organisms it is impossible to compare them with those found in the present work. It is possible that the organism designated in this paper as 506 is identical with Goldsworthy's fluorescent type. The organism designated 357, however, does not appear to be identical with Goldsworthy's second type, inasmuch as the former produced a fluorescence in certain media, while the latter is reported to be nonfluorescent.

With the information at hand regarding *Ps. cerasi* and according to the general concepts of species limits, Wormald was probably justified in creating a new species. In view of the situation set forth in this paper, however, the writer hesitates to accept it as a new species until further work has been done to prove its relationship to *Ps. cerasi*.

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CORYNOSE TWIG BLIGHT OF THE AMERICAN BLADDER NUT, STAPHYLEA TRIFOLIA

W. H. DAVIS

For the past three growing seasons, blighted twigs bearing fruiting bodies of a Coryneum have been observed on shrubs of the American bladder nut, *Staphylea trifolia* L. Since no reference to this disease was found in our best host indexes and available literature, an investigation was begun in the spring of 1930 with a view to answering the following questions:

1. Is this Coryneum the pathogen causing the twig blight?
2. How prevalent and injurious is the disease?
3. When and where does infection occur?
4. What is the Latin binomial of the fungus?
5. Is there an ascogenous stage?
6. From a study of the life history of the fungus, what methods of its control can be recommended?

SYMPTOMS AND SEASONAL OCCURRENCE

The first symptoms of the disease resulting from new infection during the current year were observed in August. These symptoms were a dying of the tips of the youngest twigs or the ashen gray color of the nodes. Later, however, lower nodes on infected twigs assumed a cartridge buff, the young buds died or failed to appear, and areas at internodes often assumed a cinnamon rufous hue. The infection sometimes advanced to a node where the Roman green color of the healthy bark could be easily differentiated from the buff of the infected area (Fig. 1, I). At this point, the tissues in the diseased area often contracted and wrinkled so as to leave a raised girdle around the twig between healthy and diseased tissues. The girdle appeared as a collar varying from a fine line, 0.5 mm. to 5 mm. in width (Fig. 1, I, 3). Furthermore, the organism, which generally entered at the nodes, advanced along both the longitudinal and radial axes of the stem but its longitudinal advance generally was halted at branches or at the beginning of the current year's growth. Furthermore, the organism advanced from younger twigs into older ones that often died during or at the close of the growing season. The disease appeared to advance during early winter, when more diseased twigs were observed than at the close of the growing season. As a final result, 30 per cent of the current year's twig growth was killed on some shrubs.

In 1930, the fruiting bodies or acervuli were first observed on newly infected wood during September. Acervuli formed around that portion of the twig receiving the initial infection or near the node. These acervuli,

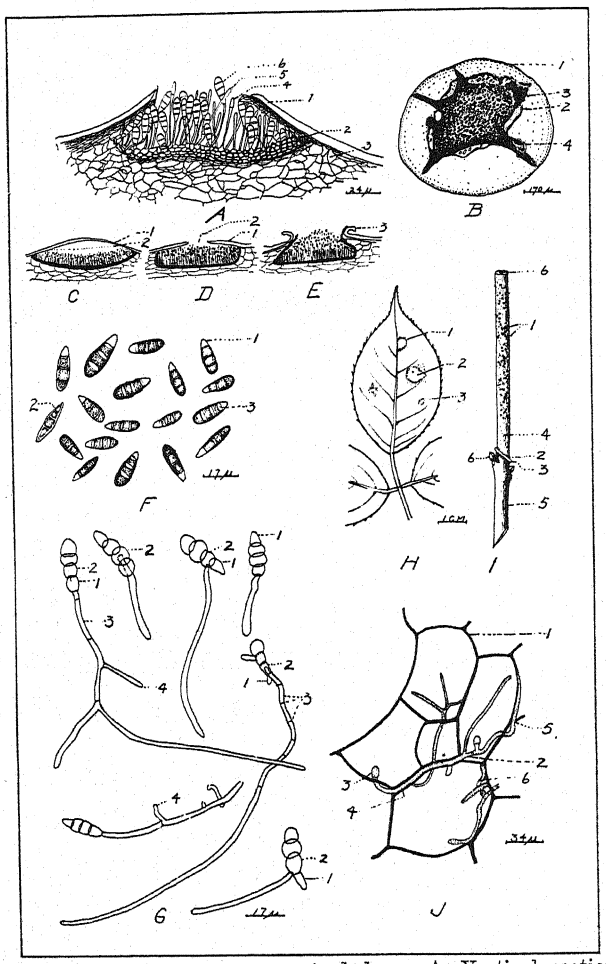


FIG. 1. *Coryneum microstictum* var. *staphyleae*. A. Vertical section of an acervulus; 1, epidermis of the host; 2, sclerotial cells; 3, cortical tissue; 4, immature conidium; 5, conidiophore about to form a conidium; 6, mature conidium. B. Acervulus on bark of host; 1, margin of the brown, "oily"-appearing bark; 2, recurved epidermis; 3, conidia; 4, rift in the bark. C, D, E. Vertical sections of acervuli; C, saucer-shape or flat before rupture of the epidermis; D, with epidermis ruptured; E, the long conidiophores projecting through the rupture make the acervulus appear conical. F. Conidia; 1, 2, hyaline pedicellate cell; 3, septum. G. Conidia germinating in water; pedicillate cell, 1, not germinating, but with cell next to it; 2, germinating and pushing it aside; 3, cross walls; 4, branches. H. Leaflets; 3 weeks after inoculation; 1, 2, 3, circular lesions. I. An infected twig; 1, acervuli; 2, limit of diseased bark; 3, collar between healthy, 5, and diseased bark, 2; 4, wrinkles in the diseased bark; 6, node. J. Pith cells from diseased twig hand-sectioned and stained with lacto-phenol containing acid green: 1, cells; 2, mycelium; 3, haustorium; 4, intercellular hypha; 5, hypha penetrating a cell wall; 6, intracellular hyphae.

when observed by aid of the hand lens, appeared as black, slightly raised, circular areas averaging 0.5 mm. in diameter, arranged singly but numerous and in close proximity or gregarious (Fig. 1, I). Bark peeled from 1 cm. along the longitudinal axis of a diseased twig contained 158 acervuli.

Acervuli that had remained in diseased twigs during the winter often appeared ashen gray and bore longitudinal rifts through the center (Fig. 1, B, C, D, E). However, some acervuli raised the epidermis of the host and might have been mistaken for the beaks of perithecia. Sometimes, small areas around the rifts of acervuli and the surface of the twig were an ashen gray, while the acervuli were of a red (tawny) color, which often disappeared as the growing season advanced. This change of color was most noticeable in old stems that had become infected by the fungus advancing from younger twigs.

Typical springtime symptoms on twigs of the previous year's growth were ashen gray bark with shrunken surface in which were slightly raised, black, gregarious acervuli. Matured acervuli were somewhat elliptical in shape, with longitudinal slits through which the honey-color spore masses could be observed.

Acervuli were also observed on infected, dried wood exposed by the shrinking and withdrawal of the diseased bark.

Conidia were formed during late summer and early autumn in the majority of the acervuli examined. Here they passed the winter and during the springtime (April and May), the epidermis of the host covering the acervuli cracked and the conidia were exposed to the air for dissemination. Few of the conidia, formed in the autumn of 1929 and 1930, germinated during those autumns but 90 per cent germinated during the spring (March, April, and May) of 1930 and 1931 (Fig. 1, G).

PATHOLOGICAL ANATOMY

Hyphae of the specific Coryneum involved in this study were located in medulla, xylem, and cortex of infected stems examined during the late spring and summer. Hand sections showed that hyphae in the pith were both inter- and intracellular (Fig. 1, J) and that they often traversed the medullary rays. Hyphae advanced nearly 8 inches in one inoculated twig during 8 months of incubation. Hyphae also were located throughout the pith of "water sprouts" that had grown 2 feet in length during the current year. This, however, might have been due to hyphae advancing from several and not from one nodal infection.

During the winter of 1930–1931, experiments were performed to determine whether mycelium remained viable within wood of the American bladder nut or only the conidia survived the northern-winter conditions.

Young twigs infected during 1930 and infected twigs of various ages were removed from shrubs during November, December, January, February, and March. Each of the five lots were disinfected with formaldehyde and washed in sterile water and portions of tissues removed from pith, wood, and bark of both the diseased and the healthy wood near, remote, and at the last visible point of infection. These portions were transferred to potato-dextrose agar under aseptic conditions and incubated at 20° C.

The results showed that the hyphae remained viable in nearly all the diseased twigs during November; in 10 per cent of the old twigs during December and January. Viable hyphae were also cultured from old twigs collected in February and March.

Thus, the hyphae of the organism remained viable, during the winter of 1930, in the older twigs of the bladder nut but not in young ones including the water sprouts.

ISOLATING AND CULTURING THE FUNGUS

Single conidia were isolated on April 25 and monosporous cultures were incubated at 22° C. on potato-dextrose agar in Petri dishes and test tubes. Furthermore, transfers of the fungus were made on steamed rolled oats and yellow corn meal in 500 cc. flasks.

The spores germinated readily and the fungus grew rapidly on each of these three media. Acervuli containing viable conidia were observed in the flask cultures on corn meal in 18 days and on rolled oats in 32 days after the transfers were made.

At first, the mycelium growing on potato-dextrose agar was a grayish white; after 3 to 5 days it changed to a light gray, then became a flesh color, rufous or chestnut brown, but finally became a dark sepia or black when old.

The growth was somewhat strict and rugose and formed a pellicle on which were acervuli. Acervuli were most numerous on the sides of the flask cultures facing the sunlight. The fungus grew faster and sporulated sooner on steamed corn meal, which was considered the best culture medium tested. The culture on steamed rolled oats, however, remained viable for 9 months, the longest period observed.

On May 14, the buds on *Staphylea trifolia* had formed young leafy stems 4 to 6 inches long. Young twigs and leaves in this condition were inoculated with a composite suspension of spores removed from three monosporous cultures, together with a small amount of mycelium. The inoculations were performed on shrubs outdoors and the inoculum placed on (a) punctured stems at leaf axils; (b) unpunctured stems at leaf axils; (c) punctured internodes and twig tips; (d) unpunctured internodes and twig

tips; (e) leaves, punctured and nonpunctured; (f) checks, punctured and nonpunctured stems and noninoculated leaves.

The surface of the twigs was sterilized with an 8 per cent aqueous solution of formaldehyde, washed with sterile water, inoculated, atomized with sterile distilled water, and then covered with oiled bags containing a wad of sterile, wet cotton. After 2 weeks, the bags were removed. Furthermore, to determine the time or season during which infection occurred, inoculations were made later in the season, when a majority of the spores were discharged from the host and the plant tissues inoculated were more matured. The date chosen was July 22.

During the following November, acervuli in some inoculated twigs contained conidia sufficiently matured for recognition of the fungus.

From the results recorded in table 1, it is to be noted:

1. The fungus was pathogenic.

2. Primary twig infection occurred in immature wood, at the leaf axils or nodes and at the tips or in meristematic tissues.

3. Infection did not occur at the internodes, even when they were punctured. Further observations showed that the inoculating wounds were generally healed without the symptoms of the disease appearing during the current year.

4. Leaves seldom, if ever, became infected while attached to the plant.

5. Puncturing was not necessary for infection.

6. Meristematic stem tissues, under favorable conditions, were most susceptible to infection.

Furthermore, incised leaves also were inoculated. The surface of the leaflets was sterilized and washed in sterile distilled water, and the leaves placed in Coplin breeding jars, lined with damp filter paper. The inoculum consisted of both spores and mycelium removed from each of three flask cultures and mixed with sterile distilled water. This inoculum was spread on the leaflets as follows: upper and under sides; punctured and nonpunctured surfaces; and at midrib, base, tip, and margin. The inoculated leaflets, together with the checks, were incubated at 25° C. After 2 weeks, small circular ash-color lesions, bordered by wine-color margins, appeared on two of the inoculated areas (Fig. 1, H). After 4 weeks, some of the inoculated leaves turned a brownish black and rotted, while the checks were intact but chlorotic. In no case were acervuli or conidia observed on these inoculated leaves. This experiment was repeated. Only growing twigs were substituted for the leaves and their cut surfaces were set in sterile, distilled water. Six weeks after inoculation, acervuli and conidia formed in the bark of the inoculated twigs. These two experiments, together with field observations, seemed to give evidence that twigs more than leaves favor the entrance and development of the parasite. This was

TABLE 1.—*Tabulated results for the inoculations of Staphylea trifolia with Coryneum microstictum var. staphyleae*

Part inoculated	5–14–30			5–20–30			7–22–30		
	Number inoculated	Infection		Number inoculated	Infection		Number inoculated	Infection	
		Plus	Minus		Plus	Minus		Plus	Minus[d]
Internodes of punctured stems	4	0	4	7	0	7	15	0	15
Internodes of nonpunctured	4	0	4	7	0	7	15	0	15
Tips of twigs, punctured	4	3	1	5	5	0	15	0[b]	14
Tips of twigs, nonpunctured	4	3	1	5	4	[b]	15	1[c]	14
Punctured leaf axils	4	4	0	8	7	1	15	0	15
Nonpunctured leaf axils	4	3	1	8	6	2	15	0	15
Punctured leaflets	6	1[a]	5	0	0	0	0	0	0
Nonpunctured leaflets	6	0	6	0	0	0	0	0	0
Stems and leaflets; checks	0	[b]	5	2	0	2	2	0	2

[a] No sporulation was observed.

[b] Specimen lost.

[c] Latent infection advanced upward from a diseased twig.

[d] Infections may appear after incubating the fungus for more than 1 year.

probably due to the large amount of pith in stems for $\frac{4}{5}$ of the 5 mm. diameter of young twigs is occupied by pith (Fig. 1, J). Here, the fungus seemed to meet with very little resistance in advancing along the longitudinal axis and obtained food sufficient for its healthy growth, since the pith cells were filled early in the season with stored nutrients that were apparently available to the hyphae.

The hyphae overwintered in the nodes and, in the spring, advanced into the buds or downward into living parts of the stem. The cells of the epidermis were seldom invaded but appeared in hand sections as a somewhat homogeneous layer above acervuli. During the growing season, however, hyphae seemed to advance through all parts of young twigs once they were infected.

MORPHOLOGY

Conidia. Over 85 per cent of the conidia observed were clavate or pyriform, while others were fusiform or elliptical. Ninety-eight per cent of the matured conidia were triseptate and few or no spore-wall constrictions were noticeable in fresh materials (Fig. 1, F). The color of the three upper cells was honey yellow or ochre yellow, while the lower or pedicellate cell was subhyaline, and, being sticky when wet, aided in anchoring the conidium (Fig. 1, F and I). Measurements of 100 conidia, removed from twigs in spring condition and mounted in water, follow: limits of variation, $5.1–6.8 \times 17–23$ μ. Standard, 6×19 μ. Few germinated in the autumn but 98 per cent germinated in the spring. One collection of April 24, stored in a living room until November 25 of the same year, germinated 40 per cent. Conidia placed in tap water at 22° C. formed germ tubes that, in turn, formed new conidia after 5 days' incubation.

Conidia in acervuli were borne acrogenously, singly on pedicels which were hyaline, filiform, and averaged 1.3×19 μ. However, some were observed as long as 30 μ (40 measured). The conidiophores in acervuli originated from a layer of sclerotia-like, polyhedral cells which overlaid 2 to 4 other layers. These cells became a Bismarck brown with age and formed a sclerotial mass; acervuli varied in shape from discoid to conical or pseudoperithecial (Fig. 1, C, D, E). Mature, nonerumpent acervuli, when measured at the surface of the host, averaged 0.6 mm. in diameter, while acervuli, in hand sections, varied at the base from 0.6 to 1 mm. in diameter and, from the unbroken epidermis of the host to the base, acervuli averaged 350 μ (Fig. 1, A).

Although the fungus was cultured for 7 months on three media of varying acidities and on twigs of the host, no perfect or ascogenous stage was observed. None of the old twigs of the host bore an ascomycete which, when cultured, produced a Coryneum. The fungus could overwinter as

perennial mycelium or as viable conidia in the host; therefore, an ascogenous stage would not be necessary for the completion of its annual life cycle.

TAXONOMY

At the present time fungi are classified according to their physiological, as well as morphological, characteristics. So, to determine definitely the Latin binomial of an unidentified form, both of these characteristics should be known. The literature at hand on the genus Coryneum yields very little information regarding its physiology, since most of the species have been established on morphological characters or according to the host from which the specimen was collected. Very few hosts other than the peach have been inoculated with species within the genus. Furthermore, descriptions of species are often inaccurate and incomplete.

Considering the fungus entirely from a morphological standpoint, it compares most favorably with *Coryneum microstictum* B. & Br., as reported by Saccardo[1] and Allescher.[2] The conidia are subpyriform, $6 \times 18\ \mu$ (Saccardo, $5–6.5 \times 15–17\ \mu$); apex, obtuse; 4 loculi, the lowermost one subhyaline, those above, honey color; conidiophores, filiform and hyaline.

These differences, however, were noted; stroma present, not obsolete; conidiophores, $1.3 \times 19\ \mu$, not $1.5 \times 20–25\ \mu$. Yet, a few were $30\ \mu$ in length, but they bore no viable conidia; Staphylea, an unreported host.

Until more information is available regarding the physiology and parasitism of the genus Coryneum the following classification for this parasite is suggested: *Coryneum microstictum* B. & Br. var., *staphyleae*. Host, *Staphylea trifolia* L., and the disease commonly known as Corynose twig blight.

CONTROLS

No definite spraying and dusting program was attempted. Smith[3] recommended a 20–20–200 Bordeaux spray for controlling *Coryneum beyerinkii* Oud., which parasitized peach trees in California. This should be applied in the fall and, again, just before the buds open in the spring.

Just as the bud scales of Staphylea opened in the spring under New England conditions, the conidia were disseminated from the old infected twigs and germinated best. So it would seem that any suitable spray applied at this time would be most effective in killing these conidia. Since initial infection takes place in meristematic tissues the spray, to be most effective, should be placed on these tissues. However, if the twigs bearing

[1] Saccardo, P. A. Sylloge fungorum. 3: 774–775. 1884.

[2] Allescher, A. Fungi imperfecti. In Rabenhorst Krypt. Flora. Abt. 7: 640–641. 1903.

[3] Smith, R. E. California peach blight. Calif. Agr. Exp. Sta. Bul. 191. 1907.

viable conidia and perennial mycelium were pruned and burned, preferably in the autumn, the greatest source of infection would be removed, and this would seem to be the simplest and most effective control method.

SUMMARY

1. The American bladder nut was parasitized by a Coryneum, which, in one case, caused death to $\frac{1}{3}$ of the current year's twig growth.

2. The disease was prevalent in the Connecticut Valley of Massachusetts.

3. The greatest injury occurred in the young twigs as a twig blight.

4. Acervuli, containing viable spores, were formed in the autumn, overwintered in twigs, and disseminated their viable conidia in the spring.

5. Inoculation experiments showed that infection occurred at the nodes and tips of the meristematic stem tissue during the spring.

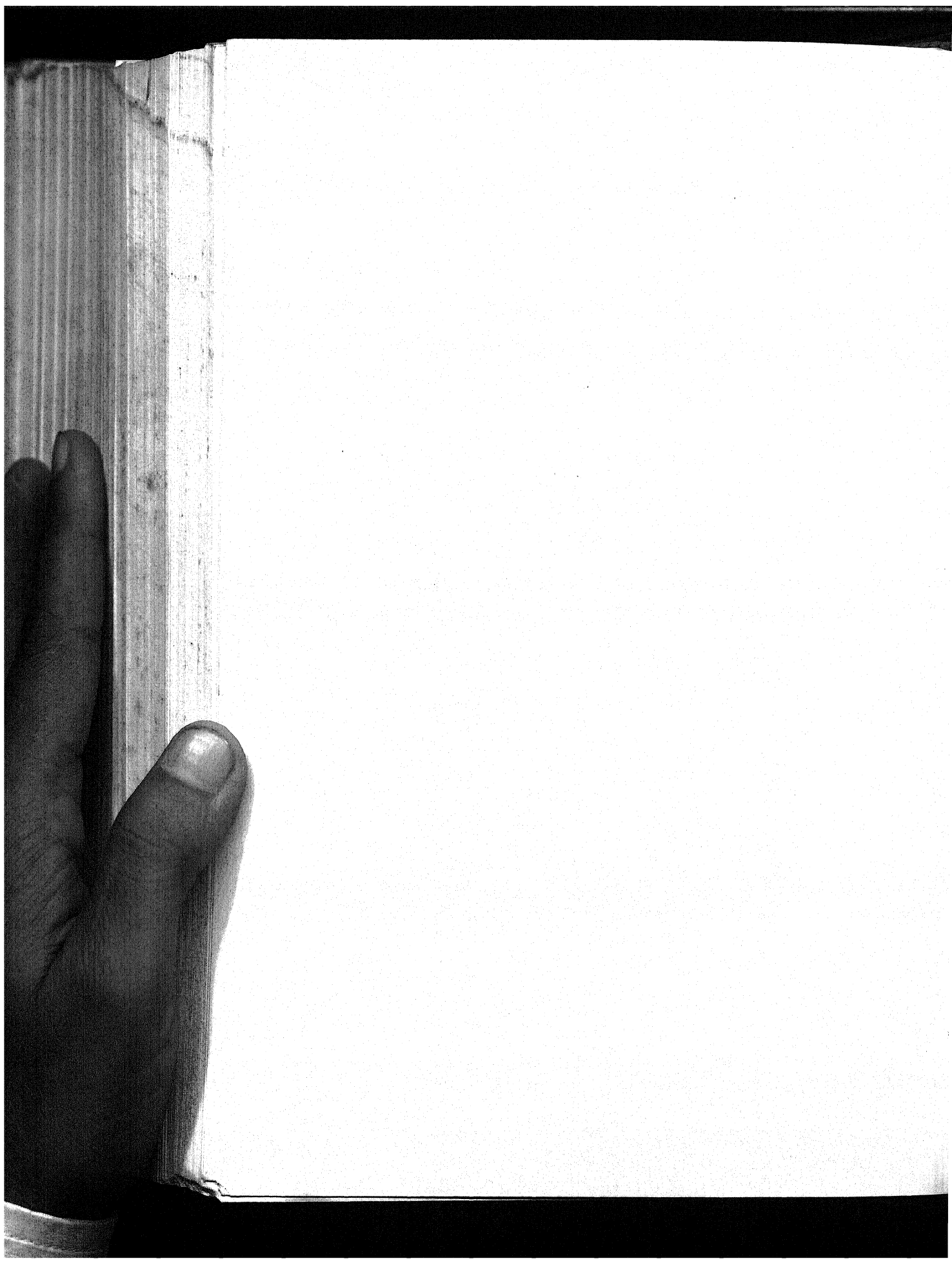
6. Mycelium of the Coryneum remained viable in some of the old stems, on shrubs, during the winter of 1930–1931.

7. *Coryneum microstictum* B. & Br. var. *staphyleae* is suggested as a Latin binomial with a varietal form; common name of the disease, Corynose twig blight.

8. No ascogenous stage was observed in the cultures.

9. To control the disease, the dead and diseased twigs should be so pruned that the cuts remove any perennial mycelium residing in them. All pruned rubbish should be burned at once, since the conidia may remain viable within incised twigs for 7 months.

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WATER BLISTERING OF WOUND DRESSINGS

RUSH P. MARSHALL

The painted surface frequently blisters when large, freshly made cuts on trees are painted thickly with tacky, impervious materials, such as heavy tar or asphalt (Fig. 1). This is particularly true when the material used does not adhere well to wet wood. While wound dressing may blister from other causes, the trouble appears for the most part to be produced by moisture, which collects between the wood surface and the protective skin of paint in the form of water blisters of variable size. In time these blisters tend to break and, so, furnish points of entrance through which insects and fungi may attack the wood.

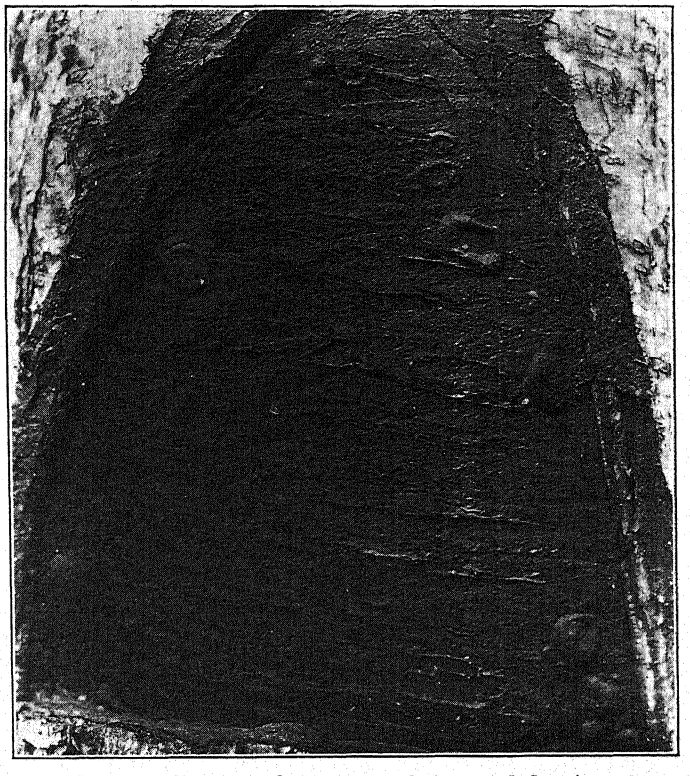


FIG. 1. Blistering of a heavy asphalt wound dressing.

In determining a method of observing something of what takes place when a cut is covered by an impervious coating, the writer is indebted to

F. A. Bartlett, who conceived a plan of covering wounds with glass. In executing this plan, both large and small glass-covered wounds were made and observed, not with the idea that they would duplicate or parallel conditions obtaining beneath the painted surface but rather in the hope that the results obtained might be indicative of what took place beneath the paint. The experiment here described was conducted at Stamford, Conn., in an open stand of mixed hardwood. The upper part of the site used is well drained but springy; while, in the lower reaches, where the birch and maple are located, the ground is wet during the greater part of the year. The trees selected for the larger cavities had a breast height diameter of 12 to 14 in., and, for the smaller cavities, a diameter of 6 to 8 in. The following tree species were included: *Acer rubrum*[1] L., *Hicoria alba* (L.) Britton, *H. ovata* (Mill.) Britton, *Betula lenta* L., *B. lutea* Michx. f., *Fagus grandifolia* Ehrh., *Liriodendron tulipifera* L., *Nyssa sylvatica* Marsh, *Quercus alba* L., *Tilia glabra* Vent., and *Ulmus americana* L.

The larger glass-covered wounds were all made very skillfully by L. Strout. The first 4 of these were put in during April, 1927, and 7 additional ones added during 1928 and 1929, making a total of 11 of the larger cavities. These wounds were made in sound wood at breast height on the trunk. They were 12 to 14 in. long, 6 to 8 in. wide, and were dug 4 to 6 in. into the wood. They were shaped as are cavities ordinarily employed by tree surgeons. In executing the work, the window glass used to cover the opening was first cut to form a lenticular pane. This was held against the trunk with its axis parallel to the axis of the tree and its outline traced on the bark with chalk. The area of wood within this line was then chamfered out to form a flat face so that the glass could be set into the opening to about $\frac{1}{4}$ in. below the cambium line. Next, leaving a $\frac{1}{2}$-in. shoulder to support the pane, the center of the area was chiseled out to form a regulation cavity. The outer edge of the cut was sealed with surgeons' tape and then covered with fitted strips cut from asphalt roofing paper. The edge and shoulder were then faced deeply with asphalt putty and the pane put into place. In this way the glass was set into a more or less flexible matrix, so as to allow slight play during the stress and strain of the trunk, occasioned by wind. The cavity was, however, made impervious to water and air. It was thus possible to look through the glass window and observe what took place behind an impervious covering (Fig. 2, A).

The callus healed well over the edge of the glass and its growth sealed the wound more and more tightly. As the callus grew it clamped down about the glass, leaving no room for play, and the pane ultimately snapped with the torsion of the trunk in the high wind or from the pressure of tree

[1] Nomenclature from: Sudworth, G. B. Check list of the forest trees of the United States, their names and ranges. U. S. Dept. Agr. Misc. Circ. 92. 1927.

growth (Fig. 2, B). In making later cavities heavy glass from automobile wind shields was substituted for window glass, but it failed to show advantage and in spite of its greater thickness, generally broke at the end of about 2 years.

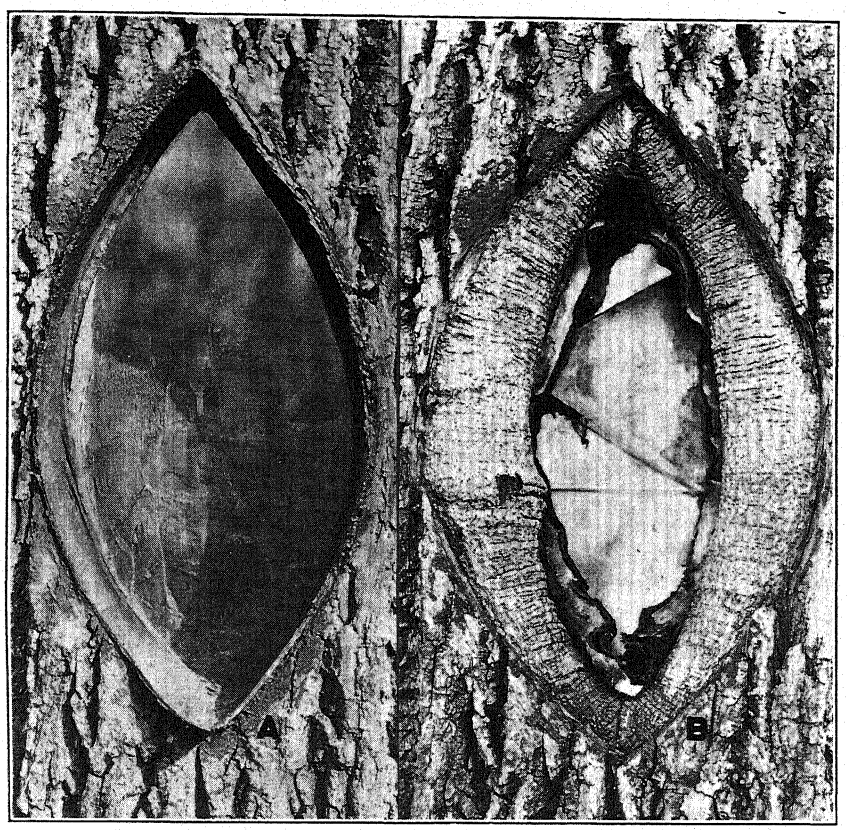


FIG. 2, A. One of the large glass-covered wounds photographed shortly after its completion. The tree is yellow poplar. B. The same wound 2 years after its completion. The callus has clamped down on the glass, and the pane has broken.

In order to permit similar observation on numerous trees without the expense of setting large glasses, or inflicting so much damage to valuable trees, an attempt was made to use small wounds covered with regulation laboratory watch glasses. These could be set within a few minutes at little cost and with a minimum of injury to the trees. They had the further advantage of permitting trees as small as 6 or 8 in. D. B. H. to be used. The modified wound was bored with a bit. It was started with an extension bit, set to cut a 2-in. hole. This was drilled deeply enough to remove

about $\frac{1}{4}$ in. of sapwood. Boring was then continued almost entirely through the trunk, using the $1\frac{1}{2}$-in. auger. The hole was then centered to the far side of the trunk with a $\frac{1}{8}$-in. twist drill. When the point of the small drill came through on the far side of the tree, the extension bit was again used to bore out another 2-in. hole $\frac{1}{4}$ in. deep, this time on the far side of the trunk. In this way a $1\frac{1}{2}$-in. auger hole passed entirely through the trunk and was faced with a shallow 2-in. hole on either side. Two-inch watch glasses were set into these 2-in. openings with either asphalt putty or grafting wax (Fig. 3, A).

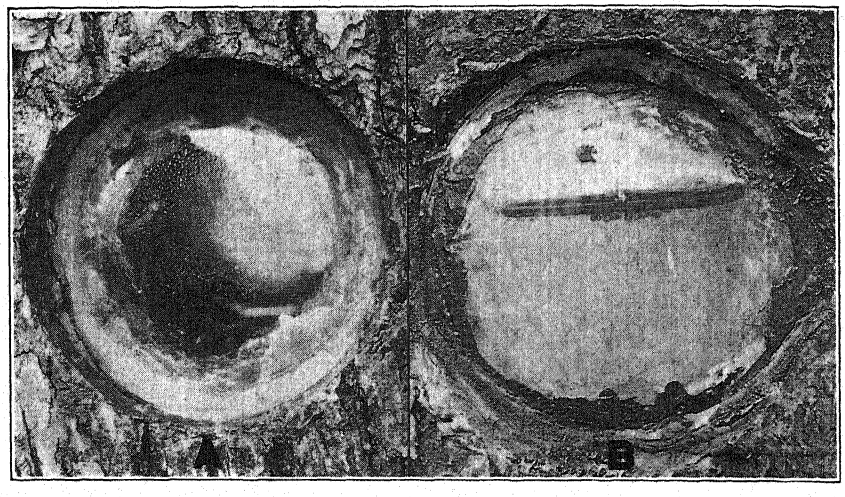


FIG. 3, A. One of the smaller glass-covered wounds photographed shortly after completion. Note the sweating of the inner surface of the glass. The wound is in white oak. B. With the rise of sap in the spring some of the smaller wounds become partially or entirely filled with liquid. The wound is in yellow birch.

Although they were easily installed, and their watch-glass windows withstood breakage well in most cases, the smaller wounds were subject to three faults, which made them far less satisfactory than were the larger cavities: (1) The material used in sealing the glasses into the openings frequently loosened and permitted the cavities to leak. (2) The cavities were too small for accurate results. (3) Close observation of what took place within them was difficult. This latter fault was evident at the outset of the experiment when in several early trials the holes were bored only part way through the trunk and the opening closed with a single watch glass. The small size of the opening and the sweating of the inner surface of the glass hindered observation. Cavities covered by a single watch glass were abandoned in favor of the method already described, because the use of two glasses admitted light, both front and rear, and facilitated inspection, particularly

when a flashlight was used to illuminate the opening from one side while observation was made from the other. Even then, the smaller cavities did not show clearly what took place at the back of the wood, since, at best, one could see only the sides of the auger hole. Despite their faults, 16 of the smaller cavities were made in 1927 and an additional 25 in 1928.

Observation of both the large and small cavities showed that moisture tended to collect behind the glass coverings. This appeared to have come from two sources, namely: (1) Sweating, due to condensation of moisture from within on the inner surface of the glass (Fig. 3, A), and (2) actual liquid that flowed from the wound. With the rise of the sap in the spring the maple and birch showed more or less profuse bleeding, while other species showed but little. In the case of the maple and birch, the watch-glass cavities tended to become partially or entirely filled with sap in the first spring following their making (Fig. 3, B). This was particularly true for both the black and the yellow birch, which bled so profusely that the watch glasses were often forced out by the pressure under which the sap flowed. In the case of the large wounds the cavities were not observed to have become more than $\frac{1}{3}$ filled with liquid. Bleeding of the red maple occurred in the middle of March, while in the black and yellow birch it did not take place until the latter part of April. In either case it was

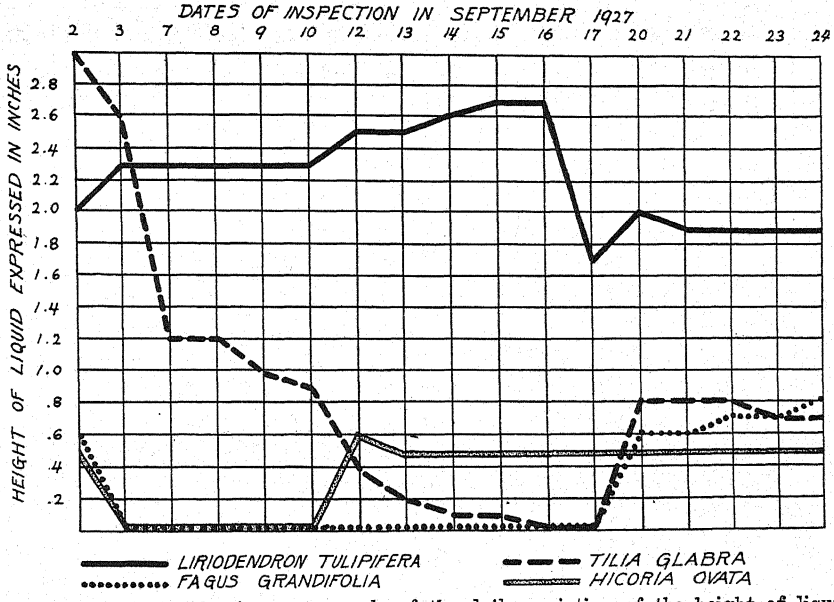


FIG. 4. Graph showing an example of the daily variation of the height of liquid which collected in the 4 original cavities made in the spring of 1927. Note that the curves lack correlation.

of relatively short duration, and within a few weeks the sap was taken up again by the wood and more or less disappeared from the cavities.

Throughout the year an unexpected accumulation of liquid was frequently evident in the larger wounds. It was so much less in volume than the spring flow that it was seldom noticeable in the smaller wounds. The volume of the liquid was found to vary from day to day (Figs. 4 and 5). Although a number of observations were made during 3 years, the writer was unable to account for the occurrence of this flow or to correlate it with either external or internal phenomena. The time it took place in some of the trees was not necessarily simultaneous with that of its appearance in others, nor did it seem to be closely connected with atmospheric conditions or with soil moisture. It took place in both the growing and dormant seasons and in both wet and dry weather.

Fungus growth occurred within several of the cavities. It was not investigated by the removal of the glasses. Observation through the glass seemed to indicate that, among other forms, Mucor, Penicillium, Graphium, Ceratostomella, and a pink form of yeast were present. Basidiomycetes were not observed.

Aside from the universally recognized function of protection against invasion of insects and fungi, no other reason is perhaps more generally given for the application of dressings to tree wounds than that of keeping out water. The well-known slogan of the painters, "Save the surface and you save all", appears to have been carried over into at least the subconscious mind of those who apply paint to trees as well as those who make use of it on houses. The scientist as well as the gardener has frequently been remiss in this regard, as is shown by the fact that articles dealing with the subject of wound dressings sometimes stress the importance of paint in keeping moisture out of the wound.

The water that collected behind the glass in these experimental cavities was much greater in amount than that occurring within the water blisters common to thick, impervious dressings. This would lead one to question the statement that one of the purposes of a wound dressing is to keep out moisture. We all know that moisture is ever present within the wood of the living tree. More than that, it is possible for active bleeding to take place at any season of the year. This bleeding is not necessarily restricted to the sapwood.

The present experiment leads us to suspect that, theoretically, the ideal wound dressings for heartwood will be a material, not too perfectly impervious, but rather one that will permit some passage of water from the wound through the dressings, thereby tending to prevent its collection be-

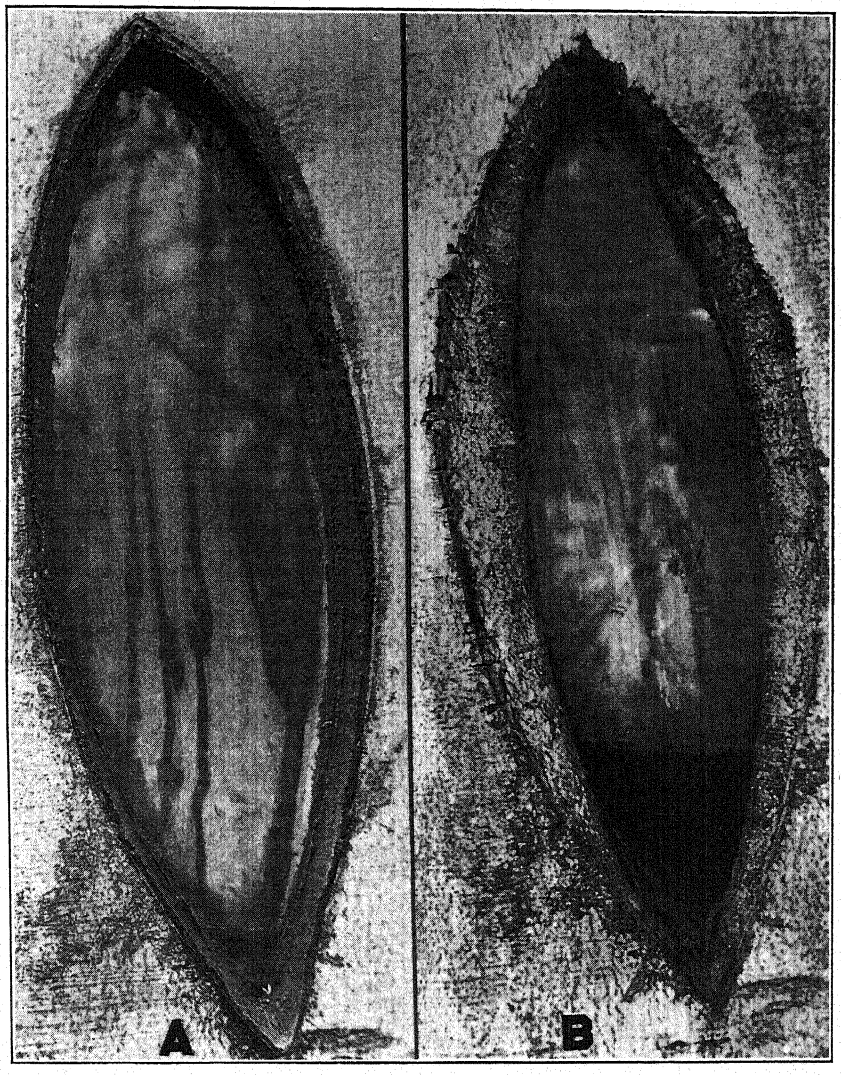


FIG. 5, A. A cavity in a beech made in the spring of 1928 and photographed shortly after completion. Note the flow from the heartwood and the collection of one inch of liquid. B. The same cavity photographed in the fall of 1929. Three and one-fourth inches of liquid were present in the cavity.

neath the surface with resultant blistering. It seems to indicate that in using any of the many excellent tree paints, which are highly impervious, we should guard against applying the material in an unreasonably thick coating.

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AGGLUTINATION STUDIES ON PHYTOMONAS MALVACEARA

O. B. Williams and H. B. Glass[1]

The present work was undertaken in the endeavor to determine whether or not there are distinct serological strains of the plant pathogen, *Phytomonas malvaceara* (E.F.Sm.) Com. S.A.B., the causal organism of angular leaf spot of cotton. A considerable amount of serological work has been done on plant pathogenic bacteria, chiefly by St. John-Brooks, Nain, and Rhodes (11), Sharp (10), Link and Sharp (7), Link and Hull (5), Link and Link (6), Link and Taliaferro (8), and Goldsworthy (1), as a result of which it has been shown that serological methods are of value in recognizing and in separating the various species. The possibility of the existence of serological strains within a single species, however, has not been the subject of any extensive amount of work.

Fourteen strains of *Phytomonas malvaceara,* isolated from field cotton plants by Dr. I. M. Lewis, in 1928, and tested for pathogenicity by him, were used for the study. These were grown at room temperature on nutrient sucrose agar, a medium that has been found especially suitable for the cultivation of this organism. It was early found that killed organisms could not be successfully used for the production of a high-titre serum. After a number of experiments with varying dosages and varying intervals of time between doses, it was found that an immune serum with a satisfactory titre could be produced in rabbits by the intravenous inoculation of living organisms. The method finally followed consisted of washing down, just before inoculation, the growth from a heavily seeded sucrose agar slant with physiological sodium chloride solution and then diluting to a turbidity approximately equivalent to 1,000 million organisms per ml. Three doses of 0.5, 1.0, and 1.5 ml. of this suspension were given at 10-day intervals, followed by a series of 3 doses of 1.0 ml. each at 3-day intervals, and bleeding 5 days after the last inoculation. The pooled serum of 2 rabbits immunized by this method had a titre of 1:6,000 for the homologous strain (No. 45). This serum was preserved by the addition of an equal volume of glycerin.

Agglutination tests with the various strains as antigens were carried out in dilutions of this serum ranging from 1:300 to 1:18,000, according to the usual methods. The tests were incubated at 45° C. for 2 hours and held over night in the ice box before recording the results. All tests have been

[1] Since this report was submitted for publication there has appeared a paper by Horgan (Jour. Bact. 22: 287–293. 1931) in which it is shown by agglutinin-absorption tests that African strains of this organism also constitute a serologically homogeneous group.

repeated at least 3 times. Considerable difficulty was encountered in the early experiments, due to spontaneous agglutination. This was finally controlled by the use of 0.45 per cent salt solution instead of the customary 0.86 per cent. A single strain of *Phytomonas bowlesii* Lewis & Wats. was included in the series, with consistently negative results. The results obtained are shown in table 1.

TABLE 1.—*Results of agglutination tests*

Strain	Serum dilution									
	300	600	1,200	1,500	3,000	6,000	9,000	12,000	18,000	Control
44	++++	++++	++++	++++	+++	++	+	+	+	−
45	++++	++++	++++	+++	++	+	−	−	−	−
46	++++	++++	+++	+++	++	++	+	+	−	−
47	++++	++++	+++	++	+	+	−	−	−	−
48	++++	++++	++++	+++	++	+	+	−	−	−
49	++++	++++	++++	+++	++	+	+	−	−	−
50	++++	++++	+++	++	+	+	+	−	−	−
51	+++	+++	++	+	+	−	−	−	−	−
52	+++	++	++	+	+	−	−	−	−	−
53	++++	++++	+++	++	+	+	−	−	−	−
54	++++	++++	++++	+++	++	+	+	−	−	−
55	+++	++	+	−	−	−	−	−	−	−
56	++++	++++	+++	+++	++	+	+	−	−	−
57	++++	+++	+	+	−	−	−	−	−	−
Phy. bowlesii	−	−	−	−	−	−	−	−	−	−

++++ = Complete agglutination.

The variations in titre obtained suggested the possibility that serological strains were present, hence absorption tests were carried out. In making the absorptions with the nonhomologous strains the suspensions were added, and, after absorption and centrifuging, reabsorption was done until all the agglutinins were removed for the strain used. This required from three to four repetitions of the process. Two strains, besides No. 45, the homologous strain, were used for these absorptions, i.e., No. 44, which the cross agglutinations had shown to be of the highest titre, and No. 55, similarly shown to be of the lowest titre. Cross agglutinations of all the strains were made with these three absorbed sera.

Although it would appear from the results given above that the organisms might be separated into at least two groups, one of high and the other of low agglutinating ability, sera completely absorbed for each of the three strains mentioned were also absorbed for each of the remaining strains, with a single exception. Strain No. 44 was agglutinated in a dilution of 1:600 of serum absorbed with strain No. 45. Since it was observed, especially with sera absorbed with strain No. 55, that, when a serum that had been

incompletely absorbed was used, differences in titre similar to those given in the tabulation were obtained, it is felt that the exception mentioned was due to incomplete absorption and is not, therefore, significant.

DISCUSSION

Tittsler and Lisse (12) have shown that agglutination in strains of *Salmonella pullorum* (Rettg.) Bergey *et. al.* is correlated with the rate of electrophoretic migration of the organisms in suspension, the higher being the rate of migration, the lower the titre obtained. This would seem to mean that in the present work the differences of titre in cross agglutinations depend upon differences in the charges on the organisms, since absorptions reveal no constant differences between them. The results here reported show, as Krumwiede, Cooper, and Provost (3) maintain, that no valid conclusions can be based on cross agglutinations alone, but that absorption tests also are necessary. Whereas earlier workers, like Hooker (2), found absorption tests confirmed cross-agglutination results, this has been gravely questioned by later workers. Differences in agglutinability may be present within strains of a single species, which are neither clear nor definite enough to provide criteria for separation of the strains into groups, as has been pointed out by Robinson (9). The work of Tittsler and Lisse (12) affords at least one explanation of these differences.

Lewis (4) has shown that strains of *Phytomonas malvaceara* are culturally quite uniform. The report here presented indicates that they are also serologically uniform.

All of the strains employed in this work appeared to be of the smooth type. The possibility of serological differences due to dissociation is being further investigated in this department.

SUMMARY

It was found possible to produce a high-titred agglutinating serum for *Phytomonas malvaceara.*

Cross reactions with heterologous strains of the same organism showed considerable differences in agglutinating titre, but absorption tests failed to establish any fundamental serological differences between the strains used.

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AN ANTHRACNOSE OF THE JUJUBE[1]

J. J. TAUBENHAUS AND WALTER N. EZEKIEL

The jujube, *Zizyphus jujuba* Mill., has been grown in Texas more than 50 years, chiefly as an ornamental.[2] The recent introduction of improved varieties, however, has encouraged planting the jujube for its fruit. Jujubes are adapted to a variety of soils, and a crop is set, even in dry years.

In August, 1926, a heavy premature shedding of the fruit was noticed in a jujube orchard near College Station, Tex. (Fig. 1, A). It was at first thought that the shedding might have been caused by the dry weather. However, some of the fruits on the ground were examined and found to be studded with numerous small, dark superficial spots (Fig. 1, D). Immature as well as ripe fruits, still clinging to the trees, were similarly affected but the foliage and limbs appeared sound. The spots first appeared as small, round, dark dots; developing into smooth or slightly sunken circular spots from 4 to 6 mm. in diameter (Fig. 1, B and C). In cases of heavy infections they frequently coalesced. The fruits on the ground were found to be more generally spotted than those still clinging to the trees. Sectioning such fruit, it was found that the interior tissues had darkened (Fig. 1, E) to a deep olivaceous color and were permeated with fungus hyphae. Severely diseased fruit shrivelled and finally became mummified (Fig. 1, F).

LOSS

To determine the approximate loss from the disease, a count was made early in September of the infected fruits on 3 trees selected as representative. It was found that 40 per cent of the fruits were diseased and had fallen to the ground; 30 per cent were variously spotted but clung to the trees; while the remaining 30 per cent were apparently sound. The disease was rather scarce during 1927 and 1928, was again destructive in 1929, and somewhat less serious in 1930.

CAUSE

Usually, no spores could be found on the surface of affected fruits; but when infected fruits were held in moist chambers over night they became covered with salmon-color layers of typical Gloeosporium spores. Pure cultures of a Gloeosporium were obtained from the spores and from bits of the interior tissues of diseased fruits (Fig. 1, H). Spores from these cultures were used in the inoculations reported below.

[1] Published with the approval of the Director as Contribution No. 155, Technical Series, of the Texas Agricultural Experiment Station.

[2] Lanham, W. B. Jujubes in Texas. Texas Agr. Exp. Sta. Circ. 41: 1926.

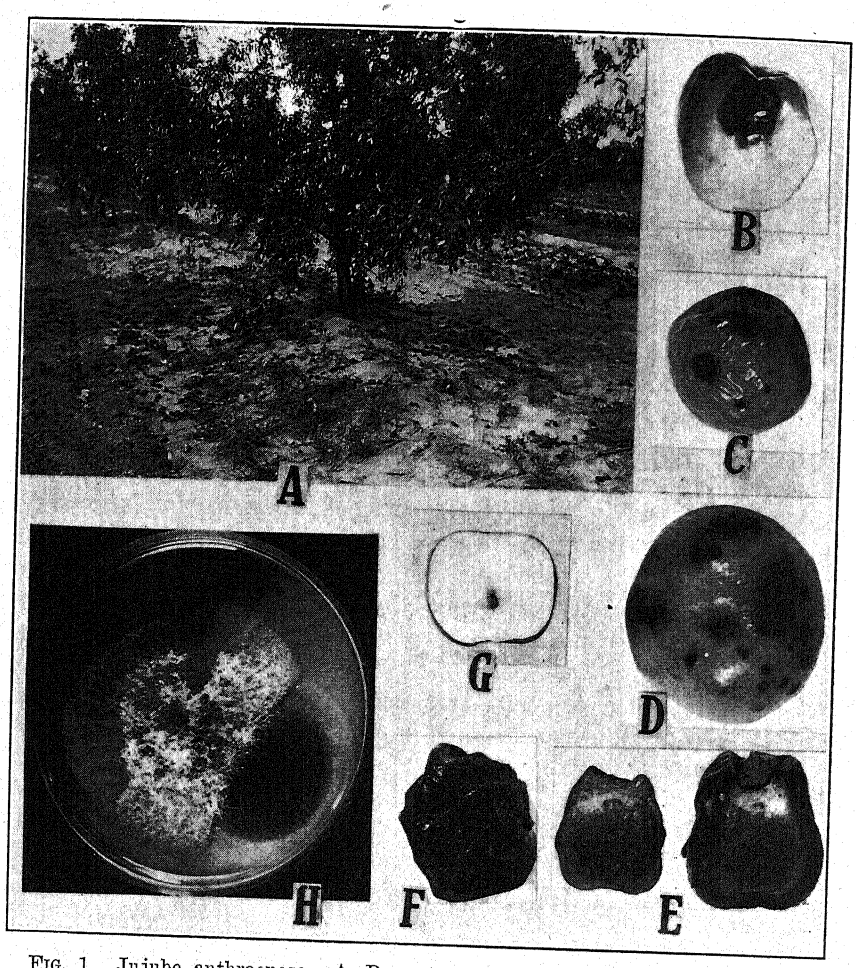


FIG. 1. Jujube anthracnose. A. Premature shedding of jujube fruit due to anthracnose. B, C, and D. Anthracnose on jujube fruits, showing variation in size of spots. E. Cross-section through infected jujube. F. Mummied, infected jujube. G. Cross-section through normal jujube. H. Gloeosporium growing from infected jujube tissue (Aspergillus contamination to the right).

On September 2, apparently sound jujube fruits on the trees were inoculated. Branches with healthy fruit were enclosed in large paper bags, sprayed with a suspension of spores, and the ends of the bags then tightly closed to prevent drying. Five hundred jujube fruits were inoculated in this way. By September 12, 86 per cent, or 432 fruits, showed definite signs of infection with symptoms resembling those found on naturally infected fruit, while only 7 per cent of 390 uninoculated fruits had developed anthracnose spots.

TABLE 1.—*Inoculations of various fruits and roots in moist chambers in the laboratory, using spores of the jujube Gloeosporium from pure cultures*

Fruits and roots inoculated	Date of inoculation	Date of observations	Results with inoculated fruits and roots			Results with check, uninoculated fruits and roots		
			Total number	Number infected	Percentage	Total number	Number infected	Percentage
	1926	*1926*						
Jujubes	Sept. 3	Sept. 15	250	108	43	250	7	3
Apples	Oct. 12	Oct. 25	25	25	100	25	0	0
Figs	Nov. 16	Nov. 22	25	21	84	25	0	0
Grapes	Oct. 29	" 4	6 bunches	6 bunches	100	6 bunches	0	0
Japanese persimmons	Nov. 16	Dec. 6	25	25	100	25	0	0
Grapefruits	Oct. 21	Oct. 29	12	12	100	12	0	0
Oranges	" 26	Nov. 3	12	12	100	12	0	0
Tomatoes, green	" 15	Oct. 19	25	25	100	.....	.....	.....
" pink	"	"	25	25	100	25	0	0
Sweet peppers	" 29	Nov. 15	24	24	100	24	0	0
Squashes	Nov. 16	" 29	12	0	0	12	0	0
Radishes	"	"	24	0	0	24	0	0
Turnips	"	"	24	0	0	24	0	0

Further inoculations in the laboratory are summarized in table 1. Sound jujube fruits were disinfected for ½ minute in a solution of 1:2,000 mercuric chloride in 25 per cent alcohol and then rinsed several times in sterilized water. Some of these fruits were then inoculated through needle punctures in the epidermis with spores from a pure culture of the jujube Gloeosporium. Similar inoculations were made on 11 other possible hosts.

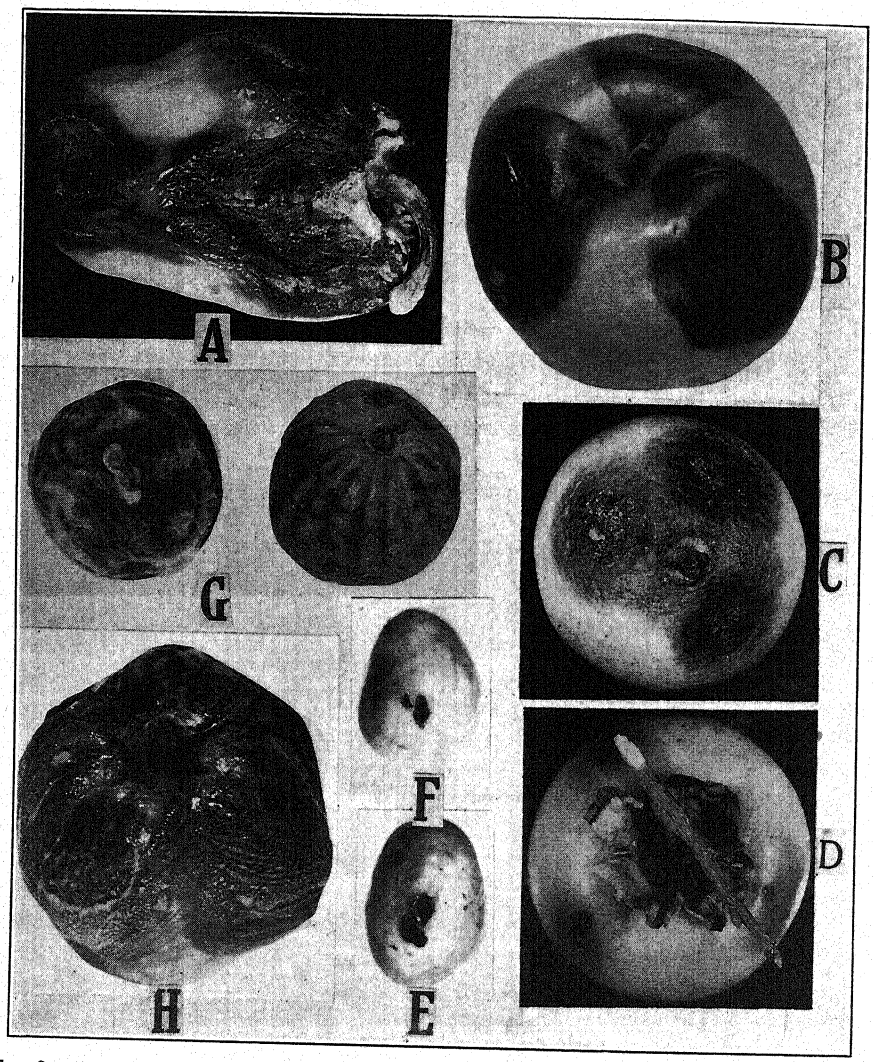


FIG. 2. Artificial inoculation of various fruits with Gloeosporium isolated from jujubes. A, on pepper; B, on apple; C, on orange; D, on Japanese persimmon; E and F, on jujube fruits; G, on fig; H, on tomato.

As listed in table 1, typical anthracnose infection was secured on the jujube fruit (Fig. 2, E and F) and on apples (Fig. 2, B), figs (Fig. 2, G), grapes, Japanese persimmons (Fig. 2, D), sweet peppers (Fig. 2, A), tomatoes (Fig. 2, H), oranges (Fig. 2, C) and grapefruit. No infection was secured with radish, turnips, or squash. From these and the previous inoculations, it appears that the Gloeosporium isolated from infected jujube fruit in the orchard is responsible, at least in part, for the jujube disease under consideration.

IDENTITY OF THE FUNGUS

On oatmeal agar, the jujube Gloeosporium formed salmon-color masses of typical unicellular spores, but no setae; while setae were numerous on jujube agar (50 gm. jujube fruits to 1,000 cc. tap water, steamed and filtered, plus 30 gm. agar). The fungus did not develop setae on artificially inoculated jujubes, figs, and apples, while they were numerous on inoculated sweet peppers and tomatoes. The fungus was grown on other media also, but no perfect stage was found there nor on overwintered mummied fruits that were examined. From the inoculations reported in table 1, however, and from the behavior of the fungus in culture, the jujube Gloeosporium probably is the same as or closely related to the Gloeosporium stage of *Glomerella cingulata* (Stoneman) S. and v. S., the cause of the bitter rot of apples, which has been shown[3,4] to attack many of the fruits that were found susceptible to this jujube fungus.

Recent isolations from diseased jujube fruit have yielded several fungi, including a species of Diplodia, in addition to the Gloeosporium mentioned, and further studies with these other organisms are in progress.

SUMMARY

A serious anthracnose disease of jujube fruit, resulting in premature shedding, was found to be caused, at least in part, by a Gloeosporium. Normal jujube fruits, as well as apples, figs, grapes, Japanese persimmons, peppers, tomatoes, grapefruits, and oranges were successfully inoculated with spores from pure cultures isolated originally from jujube fruit, and the organism was recovered from the inoculated fruit. No asci have been found on infected jujube fruit or in culture, but in general appearance and behavior the fungus appears to be the same as or closely related to *Glomerella cingulata,* the cause of bitter rot of apples.

[3] Taubenhaus, J. J. A study of some Gloeosporiums and their relation to a sweet pea disease. Phytopath. 1: 196–202. 1911.

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A DISEASE OF YOUNG COTTON PLANTS CAUSED BY SCLEROTIUM ROLFSII[1]

WALTER N. EZEKIEL AND J. J. TAUBENHAUS

Sclerotium rolfsii Sacc. is wide-spread in the South and damages a large number of hosts. This fungus has been reported on cotton plants,[2,3] which, however, have generally been considered resistant. The fungus has also been recorded as causing boll rot of cotton in Florida.[4] The present paper reports a definite disease of young cotton plants, proved to be due to *S. rolfsii.*

On July 1, 1929, cotton was planted in experimental plats at College Station, Tex., in a field of Lufkin fine sandy loam. Following unusually wet weather early that summer, some of the seedlings were found dying apparently from the attack of *Sclerotium rolfsii,* and by July 28 many plants were dead. Before the plants were thinned, the fungus spread along the rows often for distances of more than 1 foot, in one instance killing 18 plants in a space of 9 inches along the row. This severe attack, limited to restricted areas in the rows, was characteristic of the disease. Slow spread continued until about August 20, when it was checked possibly by the prolonged dry weather, although the plats were watered frequently.

Individual infected plants were readily recognized (Fig. 1, a to d). The fungus attacked and girdled the base of the stem; this was followed by rapid wilting and death of the plant. The infected part of the stem appeared water-soaked at first and then became constricted at about the ground level. The cortex occasionally split along the constricted area. Even after the upper part of the plant wilted and died, the roots remained unaffected and apparently normal.

The fungus traveled from plant to plant superficially on or through the soil. The mycelium about the base of freshly infected plants was white, usually mat-like, and readily visible to the naked eye, and sclerotia characteristic of *Sclerotium rolfsii* were produced usually by the time the plant died. The sclerotia developed superficially on the base of affected plants and on the soil (Fig. 1, a); they were spheric in shape and at first white, becoming yellow and then brown with age. Some plants did not show the

[1] Published with the approval of the Director as Contribution No. 156, Technical Series, of the Texas Agricultural Experiment Station.

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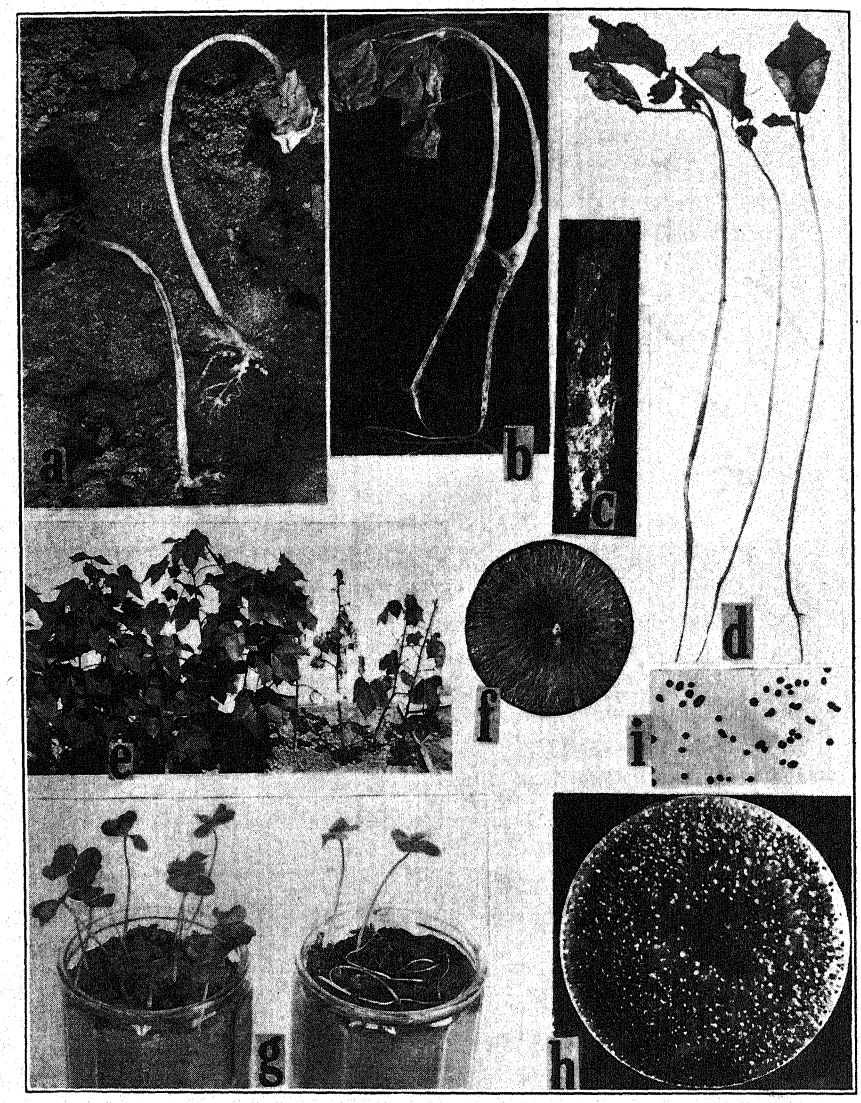


FIG. 1. *Sclerotium rolfsii* stem rot of cotton seedlings: a, two seedlings artificially inoculated with *S. rolfsii;* b and d, naturally infected seedlings; e, enlarged view of one of the plants from b; e, a mature cotton plant dying from natural infection by *S. rolfsii;* f, young culture of *S. rolfsii* from a single sclerotium from i; g, right, seedlings artificially inoculated with *S. rolfsii,* and, left, uninoculated plants; h, pure culture of *S. rolfsii* isolated from an infected cotton seedling; i, mature sclerotia from h.

strands on the surface of the stems in the field but produced a copious growth of mycelium and sclerotia when placed in moist chambers.

INOCULATION

Cultures made from surface-sterilized tissue from diseased stems, as well as cultures from the sclerotia, yielded growth typical of *Sclerotium rolfsii* (Fig. 1, h) and were similar to cultures of this fungus isolated previously from infected carrots.

Strains of *Sclerotium rolfsii* from cotton and carrots were grown on sterilized cotton stems and used in inoculations of cotton plants in the laboratory. Twelve containers filled with sterilized soil were planted each with 12 cotton seeds previously delinted with sulphuric acid and surface-sterilized in mercuric chloride solution. After the plants were up, plants in 4 containers were inoculated with the strain of *Sclerotium rolfsii* from infected cotton plants, and plants in 4 other containers with the strain from carrots. The containers were covered overnight with bell jars. Within 3 to 5 days after inoculation, the inoculated plants wilted and died, while the check plants in the 4 other containers remained healthy (Fig. 1, g). The symptoms produced on these inoculated plants were similar to those observed in the field, and the fungus was readily reisolated from the diseased seedlings. A second series of inoculations, of somewhat older cotton seedlings, was equally successful and proved that *Sclerotium rolfsii,* whether isolated from cotton or from carrots, may cause a stem rot of young cotton plants.

In 1928, several attempts had been made to inoculate mature cotton plants in another field, in connection with other experiments. These cotton plants were fully grown and had already produced well-developed bolls. The *Sclerotium rolfsii* strains used were isolated originally from carrot and from guar, *Cyamopsis tetragonoloba* L. Fresh cultures on sterilized cotton stems were placed next to the base of the plants, covered with moist straw, and water applied at intervals. No infection resulted. It is to be noted, however, that a mature cotton plant found wilting in an experimental plat in 1930 had apparently been killed by *S. rolfsii,* the only organism isolated from it in cultures (Fig. 1, e). These experiments suggest that *S. rolfsii* probably attacks older cotton plants only rarely, although it may destroy young seedlings or plants ranging from a week to 10 weeks old.

EFFECT OF SOIL DISINFECTANTS

The experimental plats in which the disease was found were being used in tests of the efficiency of various soil disinfectants for the control of Phymatotrichum root rot. On August 19, 440 plants dead or dying from

the attack of *Sclerotium rolfsii* were counted in the 30 plats of this field. There were some affected plants in most of the plats; however, there appeared to be significantly fewer diseased plants in the plats in which various organic-mercury compounds had been incorporated into the surface soil than in adjoining check plats. This was the case with K–1–X, PMA, Semesan, No. 664, and Bayer Dust. There were 275 diseased plants in 7 check plats, each about 27 feet long and 20 feet wide, while only 63 affected plants were found in the 14 treated plats, each 20 feet long and 20 feet wide. More plants with stem rot were found in each check plat than in both the adjoining treated plats. On the other hand, in the 6 plats treated with other materials, such as copper sulphate, copper carbonate, and iron sulphate, there was no consistent decrease of infection in the treated plats, which in some cases had more diseased plants than in the corresponding checks. Stucky[5] has previously reported promising results obtained by Higgins with Du Pont K–1–P applied about the base of pepper plants attacked by *S. rolfsii*.

SUMMARY

A stem rot of young cotton plants was found to be due to the fungus *Sclerotium rolfsii*. This was proved by artificial inoculations of young seedlings grown in sterilized soil in the laboratory. Artificially inoculated mature plants, in the field, however, did not become infected. Fewer diseased plants occurred in field plats in which organic-mercury compounds had been applied than in the check plats.

[5] Stucky, H. P. Director's report. Ga. Agr. Exp. Sta. Ann. Rpt. 41: 24. 1928.

A SCLEROTINIA LIMB BLIGHT OF FIGS[1]

J. J. TAUBENHAUS AND WALTER N. EZEKIEL

During September, 1926, a serious limb blight was found in many orchards in Galveston County, Tex., where the Magnolia fig is grown extensively for canning purposes. Growers claimed that the trouble was the result of the previous severe winter. However, the affected limbs died too late in the season for winter injury; there were no deep cracks in the bark of the limbs or main trunks, which characterize frost damage; and several growers stated that they had seen a similar blight in previous years, in seasons following mild winters.

The disease became noticeable as a sudden wilting of the foliage, followed by dying of the affected branches (Fig. 1, A). Other limbs were gradually involved until large portions of the trees were affected. The trunks of some trees also were attacked. More rarely, the base of a tree was infected and girdled. Examination of the diseased areas showed that they were of a water-soaked appearance and overrun by a thick, white fungus growth. The exterior and interior of the affected limbs soon became studded with numerous sclerotia (Fig. 1, B to E), which were found to resemble sclerotia of *Sclerotinia sclerotiorum* (Lib.) Mass.

Search was made in several orchards to locate the source of infection. In the vicinity of infected trees, partly buried sclerotia were found bearing mature apothecia, which were discharging clouds of ascospores. The apothecia, asci, and ascospores closely resembled those of *Sclerotinia sclerotiorum*. A culture from the ascospores was used successfully in the inoculation experiment referred to below. It therefore seems probable that ascospores constituted inoculum for natural infection.

Pure cultures were secured from bits of tissue of affected fig limbs, from sclerotia found on diseased fig limbs, and from ascospores. Cultures of *Sclerotinia sclerotiorum* were available also from celery, beans, lettuce, and snapdragons. These 7 strains of the fungus were grown on sterilized bean pods, and were used in October, 1927, in the inoculation of normal, 2-year-old potted fig plants growing in 12-inch pots. These plants were from rooted cuttings secured from the nursery of the college campus and had not previously been exposed to the fungus. The inoculum was placed close to the foot of the plants and covered with a loose layer of moist oat straw. Seventy potted fig plants were inoculated, using 10 plants for each strain of the fungus, and 10 uninoculated plants were left as checks. High percentages of infection resulted from all the strains of Sclerotinia

[1] Published with the approval of the Director as Contribution No. 154, Technical Series, of the Texas Agricultural Experiment Station.

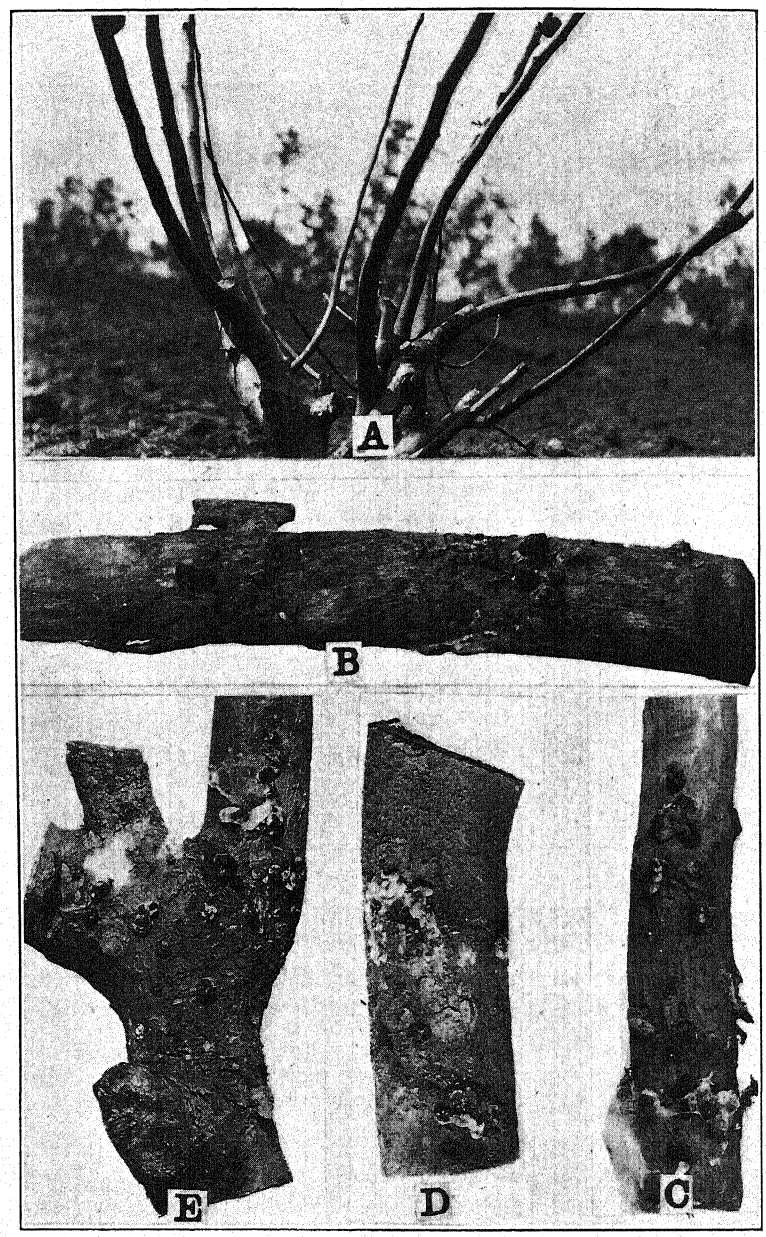


FIG. 1. Sclerotinia limb blight of figs. A. Fig tree killed by *Sclerotinia sclerotiorum*. B, C, D, and E. Blighted fig limbs studded with sclerotia of *S. sclerotiorum*.

used, while not a single infection occurred in the check plants. The appearance of the artificially inoculated plants closely resembled that of naturally-infected limbs in the orchards. The Sclerotinia was readily recovered from the inoculated plants.

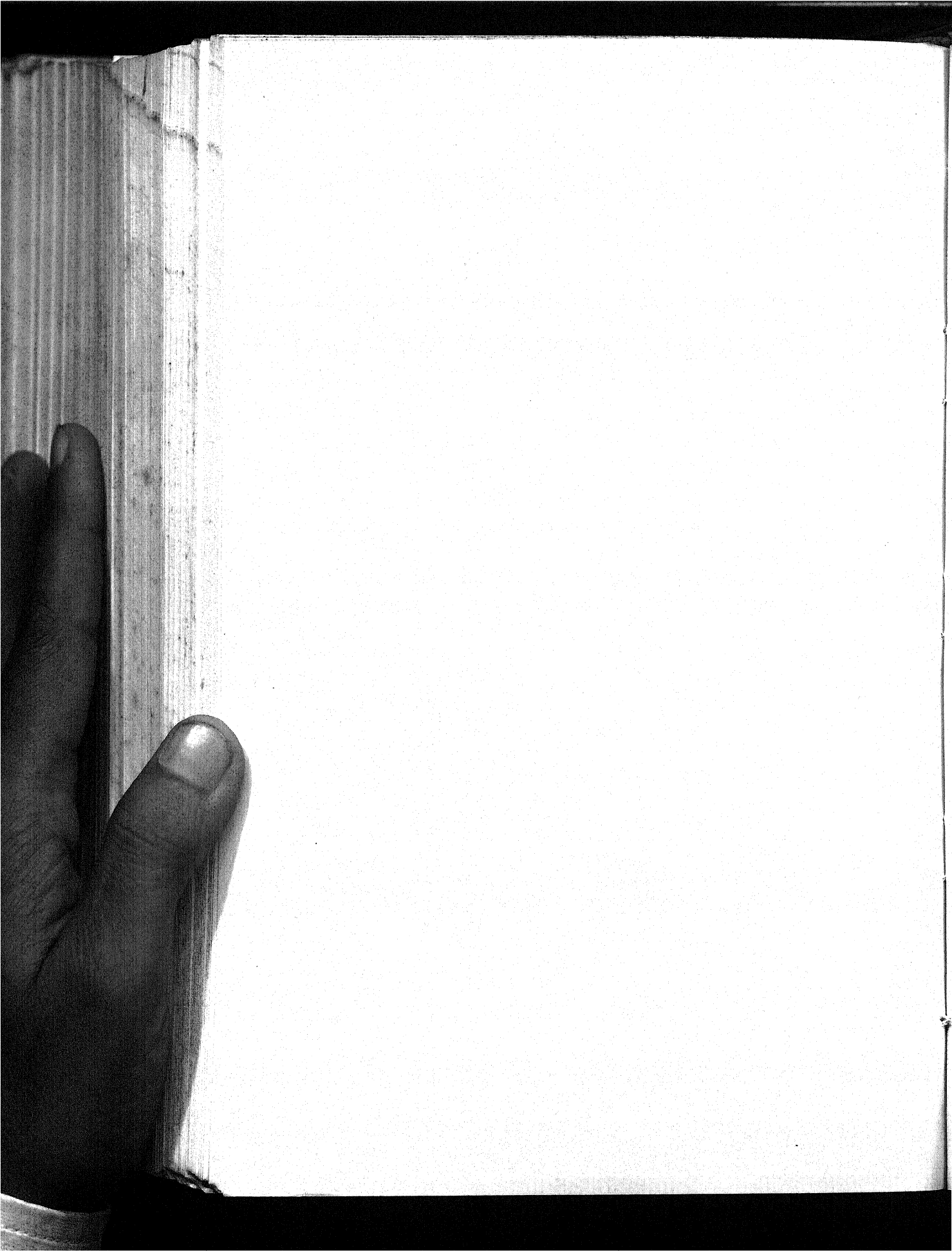
In the field blighted fig limbs, particularly the older ones, were frequently overrun by *Tubercularia fici* Edgerton. In advanced cases, evidence of the presence of the Sclerotinia was sometimes obscured, making it appear that the Tubercularia had caused the principal damage. Condit and Stevens[2] described a "die-back" of the fig in California, which they attributed to *Botrytis cinerea* and *Sclerotinia libertiana.* We found no injury from Botrytis on Texas figs, nor did the limb blight described here resemble the condition usually spoken of as die-back. *Sclerotinia sclerotiorum* appeared to be the chief, if not the only, cause of the limb blight.

Search was made to determine whether other host plants growing near affected trees in the orchards were also attacked by the fungus. Plants of *Amaranthus retroflexus,* a common weed in many of the orchards, were found heavily infected and in different stages of wilting. In other orchards, bean and lettuce plants growing in the vicinity of infected trees were also attacked by Sclerotinia. Cultures were made from infected tissues of these hosts, and the resultant growth was similar to *Sclerotinia sclerotiorum* isolated from infected fig limbs.

SUMMARY

A blight of fig limbs is described. The disease was shown to be due to *Sclerotinia sclerotiorum.* Natural infection in the field was apparently from ascospores discharged by apothecia found near infected trees.

[2] Condit, I. J., and H. J. Stevens. "Die-back" of the fig in California. Month. Bul. State Comm. Hort. Calif. 8: 61–63. 1919.



PHYTOPATHOLOGICAL NOTES

Data to be Noted in Studying Heart Rots of Living Trees.—In a rather extensive study of heart rots through a period of years the writer has found the following points of great value in studying such rots. These items are given in the hope that they may be of interest to other workers in this and related fields of research. It is not claimed that these data represent all that should be recorded, but they will give a good idea of the heart rot and what to expect under various conditions of growth, site, timber, etc.

General field observations

(1) Soil, slope, moisture, altitude, etc., where infected trees are found.
(2) Age and number of trees and species attacked by each heart rot.
(3) Diameter and height of rot in tree.
(4) Methods of entrance to tree, through roots, scars at butt, broken limbs, wormholes, etc.
(5) Position of sporophores (a) on ground, (b) on roots, (c) on trunk, (d) on branches, etc.
(6) External evidence of heart rot (a) punks, (b) hollows, (c) swelled butts, etc.
(7) Effect on growth of leaves, twigs, trunk, and roots.
(8) Odor, if any, of rot when freshly cut.
(9) Condition of rot in stump on weathering: fibrous, cubical, agglutinated, etc.
(10) In the case of conifers, infiltration of pitch, if any, in front of advancing fungus (a) in trunk, (b) in infected branches.
(11) Appearance of top of stump when freshly cut as firm, sieve-like, hollows, etc.
(12) Character of rot: wet, dry, spongy, brittle (a) when fresh, (b) when dry.
(13) Extension, if any, of heart rot into sapwood.
(14) Continuation of growth of rot in felled tree.
(15) Is the rot a root rot, a butt rot, or a top rot, or does it occur in both butt and top?
(16) Association of a given rot with other rots in same area of heartwood.
(17) Growth of rot mycelium in buried roots, humus, leaves, etc.
(18) Layers of mycelium in checks: leathery, chalky, incrusting, etc.
(19) Loss of weight in rotted wood.

Macroscopic characters of rot

(a) Early stage of rot, characteristics in radial, tangential, and cross-section views.
(b) Middle stage of rot, characteristics in radial, tangential, and cross-section views.

(c) Last stage of rot, characteristics in radial, tangential, and cross-section views.

Characters to be noted for each stage

(1) Changes in color of wood: color of rot, red, brown, white, etc.
(2) Changes in structure, as formation of holes, layers, strings, etc., or no marked changes in structure.
(3) Changes in spring wood or summer wood induced by rot.
(4) Consistency of the rot, as firm, soft, brittle, cheesy, etc.

Microscopic characters of rot

(1) Action of rot fungus on lignin, on cellulose.
(2) Action of rot fungus on cell contents, as on sugars, starches, etc., stored in wood.
(3) Action of rot fungus on medullary rays.
(4) Action of rot fungus on vessels or tracheids.
(5) Action of rot fungus on wood parenchyma.
(6) Action of rot fungus on wood fibers.
(7) Changes in structure and chemical nature of cell walls.
(8) Origin of changes, as from within outward or middle lamellae inward.
(9) Action on pits or torus of pits, on the three lamellae.
(10) Character of action on various wood elements, as marked destruction in structure visible under the microscope without staining or as shown only by using staining reagents.
(11) Holes, fissures, etc., in wood elements, due to enzymes or to action of fungus hyphae.
(12) Production of brown humus decomposition bodies and their deposit in cells.
(13) Enzymatic changes in wood in advance of fungus hyphae.
(14) How far, radially and vertically, the infecting hyphae extend beyond the visibly discolored incipient stage, determined by cultures and sectioning.
(15) Whether hyphae occur first in rays, vessels, wood parenchyma, or fibers.
(16) Along what wood elements do the hyphae travel the most rapidly? Is their movement radial or longitudinal? Do they move through the pits or bore directly through walls of the elements?
(17) Shape, size, color, location, and branching of hyphae; clamp connections and character of hyphae in the last stage of rot.
(18) Ultimate fate of each wood element for a given rot.
(19) Accumulation of carbon and ash constituents.

—W. H. LONG, Division of Forest Pathology, Bureau of Plant Industry, Albuquerque, New Mexico.

A disease of Hibiscus sabdariffa caused by Rhodochytrium.—The alga *Rhodochytrium Spilanthidis* Lagh. is known as a parasite of several wild-growing Angiosperms. Originally described by Lagerheim (1893) from *Spilanthes (Lundii?)* in Ecuador, it has subsequently been found to possess a wide host range, as well as a fairly extensive geographical distribution. Thus, it occurs in the Tropics and temperate zones of the New and the Old World on the following host plants.[1]

COMPOSITAE: *Ageratum conyzoides* L., *Ambrosia artemisiifolia* Bess., *A. trifida* L., *Solidago* sp., *Spilanthes acmella* Murray, *Sp. (Lundii?)*, *Sp. Pseud-acmella* Hook & Arn.

ASCLEPIDACEAE: *Asclepias pumila* Vail.

The purpose of the present note is to record the occurrence of an identical Rhodochytrium on a number of the Malvaceae from which family the alga in question does not seem to have been reported. The new host plant is *Hibiscus sabdariffa* var. *altissima* Wester (the s. c. roselle), which is at present cultivated as a fiber plant here and there in the Tropics.

In an experimental planting of roselle on the east coast of Sumatra (Dutch East Indies) in 1926, it was noticed by the writer that a number of individuals—about 3 weeks old—showed a peculiar, stunted appearance. The bulk of the healthy plants had at that time reached an average height of 2 feet, while the stunted ones were about 1 foot high. Only the young internodes were shortened; the older ones were of normal length. The top leaves curled slightly downwards and, moreover, showed a pronounced distortion of the surface, indicating a retardation of growth of the main vein and of some of the veinlets. These parts were found to be thickly dotted on their lower side with small, bright red galls, containing the sporangia of a Rhodochytrium, which agreed in all respects with the current descriptions of *Rh. Spilanthidis* Lagh. Both resting spores and zoosporangia were found. A number of them also occurred on the leaf petioles and on the shortened internodes of the stem.

After some time the infected plants had resumed their normal growth above the infected region, which, however, remained as an indicator of the infection. The new growth proved to be free from Rhodochytrium, probably because a period of dry weather had set in, which prevented further upward spread of the parasites to the new growth. The diseased plants did not reach the same ultimate height as the healthy plants in the same field.

It deserves to be mentioned that Rhodochytrium generally does not cause appreciable deformation of its other known host plants. Some

[1] For further details see Palm, B. The geographical distribution of *Rhodochytrium*, in Ark. Bot. (Stockholm) 18: No. 15. 1923.

species of Spilanthes sometimes do show, when infected, a somewhat abnormal elongation of internodes and petioles; in other host plants the only effect noticed is the production of the small galls. The action of Rhodochytrium on the roselle is thus the more remarkable.

As is well known, the sporangia of Rhodochytrium send haustoria-like prolongations into the fibrovascular bundles of the host tissue. The presence of the alga in these elements of a plant that, like roselle, is cultivated for its fibers, is thus clearly detrimental. So far, this disease is of no economic importance, having been found only in one field. Its occurrence should, however, be watched, as Rhodochytrium is widely spread in tropical and subtropical countries where the cultivation of roselle is most likely to be attempted.

No control measures have been necessary so far; should they become needed in the future, a scrupulously clean cultivation of the field for a short time after the germination of the seed would probably give sufficient protection. This was indicated by the fact that Rhodochytrium had infected the roselle from two of its weed host plants, *Spilanthes acmella* and *Ageratum conyzoides,* on which it occurred in great abundance in the experimental field mentioned.—B. T. PALM, Department of Botany, University of Illinois, Urbana, Ill.

A Chinese wheat resistant to flag smut.—Field studies of resistance in wheat to the flag smut organism, *Urocystis tritici* Koern., were initiated at the Agricultural Experiment Station, University of Nanking, China, in 1925, by Dr. R. H. Porter. Several Chinese wheat selections and a large number of foreign varieties were tested for their reaction to the flag-smut pathogen. These experiments have been continued up to the present time. The field experiments were first carried out by inoculating enough seed of each selection with smut spores to plant a 5-ft. row. Noninoculated seed was planted every 10th row as a check. After the first year the most susceptible selections were discarded, and the most promising resistant ones were inoculated and planted in rod rows repeated 5 to 10 times. As a result of these tests several selections of Chinese wheat were found to maintain their resistance consistently. One of these selections, now known as Nanking No. 716, has never shown any smut. It originated from a head selection collected by Dr. Porter at Weihsien, Shantung, in 1925. Because of its marked resistance, as well as its good growing habit, it was given a special supplementary greenhouse test in the fall of 1927 and again in 1928. The results of these tests, in each case, were read in the spring. The seeds were planted in 12-in. pots, filled with infected soil secured by mixing the soil for each pot with 50 gm. of smut spores. Five kernels of wheat were planted in each pot. The results are shown in the following table:

Name of wheat	1928			1929		
	Total number of plants	Number of infected plants	Percentage of infection	Total number of plants	Number of infected plants	Percentage of infection
Nanking 716	147	0.0	0.0	72	0.0	0.0
H. 1102	121	42.0	34.7	91	20.0	22.0
Nebawa	.....	.....	.....	65	0.0	0.0

Nabawa, a variety known to be resistant to flag smut in Australia, was secured from Waite Agricultural Research Institute, University of Adelaide, South Australia, and H. 1102 is a Chinese selection that has been used for checks because of its high susceptibility.

Nanking No. 716 will be increased and distributed for trial in various parts of China.—T. F. YU and H. K. CHEN, Plant Pathology Laboratory, University of Nanking, Nanking, China.

A machine for the treatment of small samples of seed grain with dust disinfectants.—In the course of the cereal-seed-disinfectant experiments at Madison, Wis., hundreds of different treatments are made for a single planting. The machine here described and illustrated speeds up the accurate and organized handling of such large numbers of small samples.

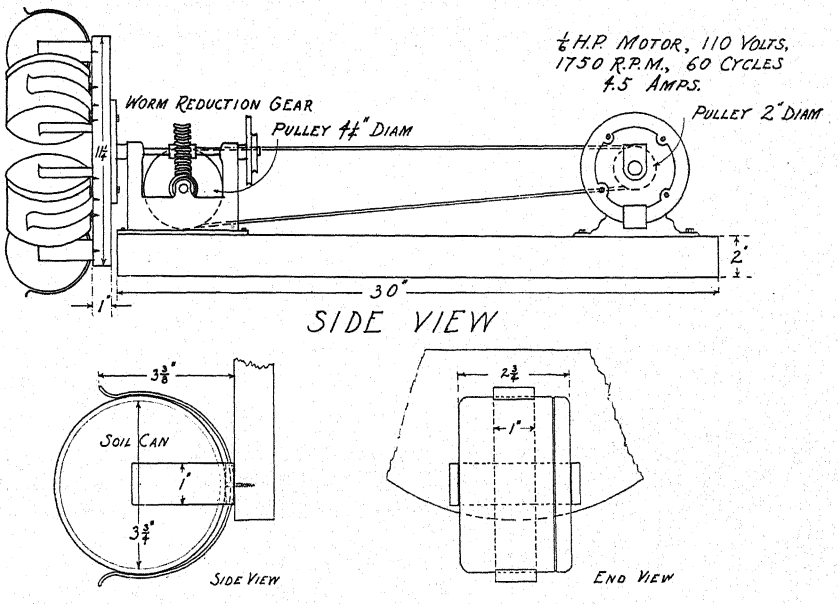


FIG. 1. Drawing showing dusting machine for treating small samples of grain.

This dusting machine (Fig. 1) consists of 6 soil-moisture cans clamped onto a revolving board by means of brass spring strips. The board is turned by a motor through the agency of a worm reduction gear, at the rate of 18 r. p. m., which speed is sufficiently slow to eliminate the cracking of grain and still fast enough so that 6 minutes' rotation thoroughly mixes a sample of grain with a dust disinfectant. Two sets of cans are used so that the operator can change and refill one set while the other set is revolving. When the seed is treated with excess dust and then screened before packeting one man can treat 60 samples of grain per hour, even when using a different disinfectant for each sample. When treating a previously weighed or measured series of samples, slightly more time is required for each treatment.

The cans filled ¾ full of grain hold 280 gms. of wheat, 215 gms. of barley, or 180 gms. of oats. When ¾ full the uniform mixing with the dust disinfectant is accomplished in a minimum of time.—W. H. THARP, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

The prevention of arsenical injury to peach twigs and foliage in Virginia.—Peach twigs and foliage are very susceptible to arsenical injury and a great deal of damage has been caused in recent years in Virginia from lead-arsenate applications. Varieties vary considerably in their susceptibility to arsenical burning. Of the commercial varieties grown in Virginia, the J. H. Hale is the most susceptible; Elberta, next; and Belle of Georgia, the least susceptible.

Hydrated lime has been used extensively by peach growers to check this type of injury, but it has proved almost worthless as a preventive during wet seasons. This has made the lead-and-lime spray a very dangerous one, but the lack of anything better has compelled the growers to continue its use. A spray combination, however, that has recently proved very efficient in preventing arsenical injury to both twigs and foliage is the zinc-lime spray introduced by John W. Roberts, of the United States Department of Agriculture, for the control of the bacterial shot-hole disease. A 4–4–50 zinc-lime formula (zinc sulphate, 4 lbs.; hydrated lime, 4 lbs.; powdered lead arsenate, 1 lb., and water, 50 gals.) was used in approximately half of the commercial peach orchards in Virginia during the season of 1931, in all of the lead-arsenate applications. In every case where the zinc-lime material was used in all of the lead-arsenate applications scarcely a trace of arsenical burning could be found, whereas, in other orchards, where this material was not used, severe defoliation occurred, even if only one lead-arsenate application was made.

It is suggested that the zinc hydroxide, which is formed by chemical reaction between the hydrated lime and zinc sulphate, takes up the water-soluble arsenic in the form of an insoluble basic zinc arsenite.—R. H. HURT, Virginia Agricultural Experiment Station, Blacksburg, Virginia.

BOOK REVIEW

The Plant Rusts (*Uredinales*). By Joseph C. Arthur in collaboration with F. D. Kern, C. R. Orton, F. D. Fromme, H. S. Jackson, E. B. Mains, G. R. Bisby. iii–v + 446 pp., 186 figs. John Wiley & Sons, Inc., New York; Chapman & Hall, Limited, London, 1929. Price, $6.50.

Any discussion of the rusts by Dr. Arthur would be interesting because of the author's unique experience and eminence and because of the scientific standing of those who collaborated with him in writing the book. But "Plant Rusts" is interesting in its own right also because it contains a wealth of important information and expresses fundamental view-points.

In the first chapter is given an elementary but clear description of rusts in general. Chapter 2 summarizes in an interesting manner the historical development of uredinology. Chapter 3, on development and classification, plunges directly into details and concepts which presuppose a considerable previous knowledge on the part of the reader. The view-points expressed in this chapter seem refreshingly sound and sensible. For example, the statement on page 80 that the origin of rust species "can be explained more fully by tracing chromosomal inheritance" and the concept that heteroecism is merely one kind of specialization must appear to progressive mycologists as sensible. Such statements are gratifyingly modern, especially as there has been a tendency in the past to surround these phenomena with a somewhat thick veil of pseudoscientific mysticism. The directness of thought and reasoning is therefore noteworthy and commendable. Some mycologists may be inclined, however, to question the statements regarding the degrading effect of parasitism, as, for example, the following statement on page 97: "The mode of life has disturbed and exaggerated the balance between vegetative and reproductive activities greatly in favor of the latter as compared with non-parasitic plants." Whether the mode of life really is responsible for the development of peculiarities in the rusts may be open to question.

In the discussion of classification it is frankly admitted that the classification of rusts attempts to meet practical requirements and does not necessarily indicate natural affinities, desirable as the latter might be. The position with respect to *nomina conservanda* will probably be welcomed by most disciples of common sense, even in mycology.

The cytological and morphological features of the rusts are well summarized and illustrated in chapter 4, although the significance of cytological phenomena is given relatively little consideration. Possibly it is as well: in the past there often has been too much speculation regarding the meaning, real or fancied, of certain phenomena.

The dissemination and geographic distribution, especially the latter (Chapter 5), are well and adequately discussed. The chapter is packed with interesting facts, and the interpretations and conclusions seem sound. In chapter 6 the phenomena of spore germination and the factors affecting germination, the subsequent development of germ tubes, their entrance into the host, and the development of mycelium and haustoria are described for a number of rusts. There is a brief discussion also of the effect of environmental factors on the development of rusts. Some pathologists may wish to improve the definition of infection given on page 231, but the term has no precisely standardized meaning, and the author of the book has at least made his concept fairly clear. A fuller discussion of the effect of environmental factors on infection and the subsequent development of the rust might have been desirable, but perhaps this would have exceeded the scope intended for the book.

Chapter 7 is devoted to a consideration of physiologic specialization and the nature of resistance and susceptibility. The section on specialization probably could have been made clearer by a more liberal use of keys and tables. The writer would be inclined to take issue with some of the statements in the discussion of resistance, but, as they are matters of opinion and interpretation, there probably is no need to go into details.

Chapter 8 contains interesting information on abnormalities and diseases of rusts, together with a description of pathologic changes caused in host plants.

Chapter 9 is devoted to a discussion of economic considerations. One obtains a good idea of the importance of the most destructive rusts of the principal groups of economic plants, although the discussions are necessarily brief. The last chapter, on methods of investigation, brings together some useful information on the collection and preservation of specimens, the technic for microscopic study, and culture methods. The index leaves something to be desired, and it is hoped that it will be amplified in subsequent editions.

The sequence of subjects discussed in the various chapters is logical, and, despite the difficulties pointed out in the preface to the book, a rather high degree of unity has been attained. The style is clear, for the most part, and, except in a few places, the statements are direct and free from ambiguities. There is some repetition, but this probably is not only unavoidable but even desirable.

The desirability of uniform terminology in mycology becomes evident in reading the book. The terms sporophyte and gametophyte are used in the book in preference to the terms haplophase, dikaryophase, and diplophase. The writer suggests that the latter are more precise and more nearly meet the requirements, especially in the rust and smut fungi. The use of

the terms physiologic race and physiologic form may lead to further confusion where much already exists. The use of "physiologic race" to denote the physiological entities such as *Puccinia graminis tritici,* while accepting the term physiologic form for the component entities, partly disregards previous usage without clarifying the situation. The statements regarding specialization are not always clear because, in at least some instances, terms are used loosely or inconsistently. For example, on page 260 one reads: "Similar situations occur in other weakly specialized rusts such as *P. graminis* and *P. coronata.*" But on page 336 the following statement is made: ". . . the species (*P. coronata*) does not show the sharp host specialization or separation into races which is shown by *P. graminis.*" Specialization probably is considered from different viewpoints in the two statements, and the result is an apparent inconsistency. The propriety of designating as "mutable species" the long-cycle rusts "which pass readily at times from the long-cycle to a condition that actually is, or else resembles a short-cycle condition" may also be questioned by those who would restrict the meaning of the word mutable to a tendency to change genotypically. These few minor defects, if they are defects, do not detract appreciably, however, from the general excellence of the book.

The voluminous literature on rusts has been well and fairly summarized. In a few instances reference is not made to papers in which important phenomena were first described; instead, later and more comprehensive papers are cited. The reader in such instances does not get the historical perspective quickly. The problem of selecting literature for citation is, of course, a difficult one, and the relative importance of contributions is partly a matter of personal opinion. It would have been natural to feature the extensive investigations of the author and his associates. This tendency, however, has been avoided and the material is presented with a high degree of objectivity.

The scientific tone of the book is excellent throughout. Controversial questions are discussed with dignity, fairness, and excellent judgment. There is a nice balance between fact and philosophy, with a soundness of viewpoint that would, of course, be expected of a man of the author's eminence and the high scientific attainments of his associates, but which nevertheless does them credit.

Botanists in general and mycologists in particular owe Dr. Arthur and his associates a debt of gratitude for bringing together in book form so many facts regarding rusts and, especially, the rich experience and broad viewpoints obtained from their own extensive and fruitful investigations.—E. C. STAKMAN, University Farm, St. Paul, Minnesota.